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James R Hunt

Papers submitted to the University of Bristol in accordance with the requirements of the degree of Doctor of Philosophy by published works

in the

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Summary

Five papers are submitted to support an application for the award of PhD by published work. These papers describe work carried out over a six-year period at the University of Bristol. The focus of all of the publications is the investigation of clinical pain mechanisms and strategies for its management in dogs and, to a lesser extent, cats in the United Kingdom. The publications employ a range of methodological approaches, as appropriate to the research question; and considered as a whole they examine clinical pain topics ranging from acute to persistent forms of pain, and from individual to population level.

On admission to the Royal College of Veterinary Surgeons, members promise and solemnly declare that "*my constant endeavour will be to ensure the health and welfare of animals committed to my care*"[1]. However, historically acute perioperative pain has been undertreated by Veterinary Surgeons in a number of countries, including the UK. Persistent ("chronic") pain in humans currently represents the one of the greatest healthcare burdens and significantly decreases quality of life in affected people yet is often poorly responsive to common analgesic interventions. Similarly, painful conditions such as osteoarthritis which cause persistent pain in people are common in dogs and cats and have been identified as one of the top three welfare issues in pet dogs. Robust examination of pain and analgesic strategies intended to manage pain in animals, and communication of the resulting findings

[1] https://www.rcvs.org.uk/setting-standards/advice-and-guidance/code-of-professional-conduct-for-veterinary-surgeons/ accessed 4th July 2018

to an appropriate audience therefore have the potential to improve welfare for a significant number of animals undergoing veterinary treatment; and this consideration has motivated the research presented within this work.

Paper 1 reports a evaluation of the analgesic efficacy of two opioid drugs with marketing authorisation for use in dogs in a highly clinically relevant model of orthopaedic surgery, within a standardised and pertinent anaesthetic management protocol. This research has contributed to an evidence base enabling, for example, the recommendation of full μ agonist opioids in preference to partial agonists for major surgery in dogs (Murrell, 2014), which would be expected to improve the provision of perioperative analgesia and animal welfare.

Paper 2 details a cross-sectional survey of perioperative analgesic prescribing practices amongst UK veterinary surgeons and draws comparisons with previous surveys. The results indicate significant improvement in the use of perioperative opioid and non-steroidal anti-inflammatory drugs for management of perioperative pain, and highlights areas such as local analgesia and postoperative administration of non-steroidal anti-inflammatory drugs to cats where prescribing could be improved.

Paper 3 considers the adverse events associated with administration of analgesics, specifically non-steroidal anti-inflammatory drugs. A number of challenges were encountered in accessing and interpreting the data which are discussed, however the work documents the state of our knowledge in respect of adverse event reporting in dogs and cats, and presents opportunities for further investigations.

Papers 4 and 5 resulted from a 3 year experimental investigation into changes in spinal cord processing of nociceptive stimuli in client owned dogs with osteoarthritis. Paper 4 details the development of a suitable technique in laboratory dogs to probe spinal nociceptive

processing under general anaesthesia – a prerequisite for humane data collection in client owned animals. Electromyographic responses to nociceptive stimuli are recorded as a quantifiable estimation of spinal nociceptive processing. Paper 5 describes the application of the technique to a population of client owned animals diagnosed with pelvic limb osteoarthritis, and documents increases in nociceptive processing in affected animals, compared to a control group of unaffected client owned dogs. Additionally, in a subgroup of animals recruited, a technique for eliciting a form of endogenous analgesia (diffuse noxious inhibitory controls, DNIC) is described in dogs for the first time, and less effective DNIC is identified in animals affected by osteoarthritis. These results corroborate previously published evidence of widespread somatosensory sensitisation in dogs affected by osteoarthritis, and further identify that one mechanism which may contribute to sensitisation is a reduced efficiency of DNIC. These findings are consistent with investigations into people affected by osteoarthritis, and thus further validate canine osteoarthritis as a potential model for the human disease.

Considered together these five papers represent a significant contribution to our understanding of the management of pain in cats and dogs.

Award of PhD by published work

This manuscript and collection of papers is submitted by James Russell Hunt, who is applying for a PhD degree based on published research.

Papers presented:

Clinically relevant differences in efficacy between licensed doses of opioid analgesics in dogs undergoing orthopaedic surgery

Paper 1 Hunt, J.R., Attenburrow, P.M., Slingsby, L.S., Murrell, J.C., 2013. Comparison of premedication with buprenorphine or methadone with meloxicam for postoperative analgesia in dogs undergoing orthopaedic surgery. Journal of Small Animal Practice 54, 418–424. doi:10.1111/jsap.12103

Nationwide survey demonstrating improvements in reported provision of perioperative analgesia by UK Veterinary Surgeons over the previous 15 years and highlighting potential for improved post-discharge analgesia prescription

Paper 2 Hunt, J.R., Knowles, T.G., Lascelles, B.D.X., Murrell, J.C., 2015. Prescription of perioperative analgesics by UK small animal veterinary surgeons in 2013. Vet Rec 176, 493. doi:10.1136/vr.102834

Interrogation of post-marketing authorisation adverse events reported to the UK Veterinary Medicines Regulator

Paper 3 Hunt, J.R., Dean, R.S., Davis, G.N.D., Murrell, J.C., 2015. An analysis of the relative frequencies of reported adverse events associated with NSAID administration in dogs and cats in the United Kingdom. The Veterinary Journal 206, 183–190. doi:10.1016/j.tvjl.2015.07.025

Development of a suitable anaesthetic protocol to facilitate recording of nociceptive withdrawal reflexes in dogs and preliminary investigations into the evocation of descending nociceptive modulation

Paper 4 Hunt, J., Murrell, J., Knazovicky, D., Harris, J., Kelly, S., Knowles, T.G., Lascelles, B.D.X., 2016. Alfaxalone Anaesthesia Facilitates Electrophysiological Recordings of Nociceptive Withdrawal Reflexes in Dogs (Canis familiaris). PLoS ONE 11, e0158990. doi:10.1371/journal.pone.0158990

Application of anaesthetic technique to investigate nociceptive withdrawal reflexes in dogs affected by osteoarthritis compared with unaffected animals and development of a paradigm to investigate diffuse noxious inhibitory control in anaesthetised dogs

Paper 5 Hunt, J.R., Goff, M., Jenkins, H., Harris, J., Knowles, T.G., Lascelles, B.D.X., Enomoto, M., Mendl, M., Whay, H.R., Murrell, J.C., 2018. Electrophysiological characterisation of central sensitisation in canine spontaneous osteoarthritis. Pain 159, 2318–2330. doi:10.1097/j.pain.0000000000001336

Acknowledgements

The work which led to these publications was performed whilst undertaking research posts within the Anaesthesia and Analgesia Research Group, Faculty of Medicine and Veterinary Science, University of Bristol, Langford; under the supervision of Drs. Louisa Slingsby and Jo Murrell, to whom I am grateful for their continued support and advice.

Funding support for the research which led to the publications was provided by Eurovet Animal Health, Elanco, Boehringer-Ingelheim Vetmedica, Biotechnology and Biological Sciences Research Council, and Zoetis.

The opportunity to work within multi-disciplinary research teams has been a valuable experience; particular thanks must go to Professor Toby Knowles for his guidance on statistical analysis, Professor John Harris for the opportunity of spending time in his laboratory undertaking training on EMG techniques, and Professor Duncan Lascelles for all of his advice and support.

Dr. Emma Love has kindly provided advice on the presentation of these papers.

Statement of contributions towards published work

Paper 1

I was responsible for designing the clinical study and completing applications for ethical and veterinary medicines regulations approval, data collection, performance of statistical analysis, and primary authorship of the manuscript. Peter Attenburrow was responsible for performing the surgeries, and Louisa Slingsby and Jo Murrell provided advice on all aspects of the study and contributed to editing the manuscript. Data and conclusions from the study were presented by Jo Murrell, head of the research group, during a number of Continuing Professional Development sessions for first opinion Veterinary Surgeons.

Paper 2

I took the lead on the design of the survey and refined it in conjunction with my supervisor, to expand on the data collected by previously published survey work in the UK. I created the online version of the questionnaire. I was responsible for transcribing the data and undertaking verification of the entries, performing statistical analysis, and authoring the manuscript. Toby Knowles provided support for statistical analysis, and Toby Knowles, Jo Murrell, and Duncan Lascelles contributed to editing the manuscript. I presented an abstract of the study findings at the Association of Veterinary Anaesthetists Spring meeting 2014 in Nottingham.

Paper 3

After initially contacting the National Office of Animal Health in writing, I presented the aims and proposed methods of the project at a meeting with the Veterinary Medicines Directorate and was able to satisfy them that the proposal, analysis, and reporting were justified and would be appropriately conducted. Commercial and government agency sensitivity around pharmacovigilance data were high, therefore negotiation with these stakeholders was a necessary part of the success of this project. I was responsible for data handling and analysis of the raw data presented by the Veterinary Medicines Directorate. I authored the manuscript, with input from Jo Murrell and Rachel Dean. I presented an abstract of the study findings at the Association of Veterinary Anaesthetists Spring meeting 2014 in Nottingham.

Paper 4

The study idea was conceived by Jo Murrell, Duncan Lascelles, John Harris, Sara Kelly, and Toby Knowles prior to my appointment on the research project. The study protocol was co-designed by myself, Jo Murrell, Duncan Lascelles, John Harris, Sara Kelly, and Toby Knowles. During the experiments Jo Murrell supervised the sedation and anaesthesia of the dogs, Duncan Lascelles instrumented the dogs and applied mechanical stimuli, David Knazovicky provided assistance, and I applied the electrical stimuli and recorded the stimulus parameters and response recording within the labchart software for all types of stimuli. I was responsible for data recording and spreadsheet entry, data analysis and authoring of

the manuscript. Jo Murrell, Duncan Lascelles, John Harris, Sara Kelly, Toby Knowles, and David Knazovicky contributed to the final manuscript.

Paper 5

This paper encompassed two investigations in an overlapping population of client owned dogs. As it was performed under the Animal (Scientific Procedures) Act 1986 (as amended 2013) I was required to attend training to obtain a personal licence. I developed the study protocols, with input from all of the co-authors. John Harris and I developed the EMG recording protocol used in the studies by identifying the latency of 'early' and 'late' EMG responses observed in the experiments described in Paper 4 and subsequently generating a macro within the labchart recording software to identify these responses following a stimulus. Helen Jenkins and Megan Goff monitored anaesthesia of the dogs, I was responsible for inducing anaesthesia and calculating infusion rates of alfaxalone, supervising anaesthesia, applying the mechanical and electrical stimuli and recording experimental parameters to correlate with the labchart software recordings. I was responsible for extracting and processing the data from the labchart software and entering this into an excel spreadsheet. I developed the models for multi-level data analysis with Toby Knowles, and subsequently performed the analyses under his supervision. Masataka Enomoto and Duncan Lascelles were responsible for severity scoring of the radiographs. I authored the manuscript with contributions from Jo Murrell, Duncan Lascelles, John Harris, Toby Knowles, Becky Whay, and Mike Mendl. A summary of the research was presented in two abstracts at the Association of Veterinary Anaesthetists Spring meeting 2017 in Manchester.

Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....

CONTENTS

1. An Introduction to Pain

Pain is currently defined by the International Association for the Study of Pain (IASP) as "*An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*" (IASP, 2014). The notes accompanying the definition state "*The inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment*." (IASP, 2014). Cohen et al., (2018) have criticised the current definition on a number of grounds, including that; i. "*unpleasant is an unsatisfactory descriptor*"; ii. that the accompanying Note on Usage states, "*Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons*", which they contend discriminates against pain conditions in which tissue damage or obvious pathophysiological cause is not readily identified; and iii. that the current definition *"ascribes a singular phenomenology (unpleasant sensory and emotional experience) to all instances of pain*". These authors have proposed a changed definition of pain: "*Pain is a mutually recognisable somatic experience that reflects a person's apprehension of threat to their bodily or existential integrity*" (Cohen et al., 2018). These criticisms of the current definition have been examined by Treede, (2018), who concluded that "*the article by Cohen et al., (2018) will have done a great service to the field if we take it as an inspiration for broadening our approach to pain assessment, but not as a redefinition of pain*."

Molony (1997) provided a working definition of animal pain as "*an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the*

integrity of its tissues; (note, there may not be any damage) it changes the animal's physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery; non-functional pain occurs when the intensity or duration of the experience is not appropriate for the damage sustained (especially if none exists) and when physiological and behavioural responses are unsuccessful in alleviating it". In terms of evaluating the presence of clinical pain in mammals this definition serves us extremely well, and further work has developed measurement scales in order to estimate the degree of pain in different mammalian species in specific circumstances (e.g. Morton et al., 2005; Reid et al., 2017) . Contrary to this position, given that pain as defined by IASP comprises both a sensory and affective component, it has been argued that without insight into the conscious experiences of animals we cannot reliably predict that they may experience pain, and that we cannot infer its presence from analysis of behavioural responses to stimuli as "*seemingly purposive behaviors in response to noxious stimuli are commonly expressed by decerebrate animals*" (Rose et al., 2014). However, self-selection of analgesics in rats (Colpaert et al., 1980), domestic chickens (Danbury et al., 2000), and zebra fish (Sneddon, 2013) subject to inflammatory or nociceptive conditions strongly suggests some central integration of nociceptive stimuli resulting in behavioural choices to ameliorate the aversive condition, and may provide insight as to the animals' subjective experience (Sneddon et al., 2014).

Pain may have a number of origins, including *nociceptive pain* ("*arising from actual or threatened damage to non-neural tissue and due to the activation of nociceptors*"), *nociplastic pain* ("*arising from altered nociception despite no clear evidence of actual or*

threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain"), and *neuropathic pain* ("*pain caused by a lesion or disease of the somatosensory nervous system*") (IASP, 2014). Undoubtedly the ability to perceive, respond, and alter motivational state in the presence of noxious stimuli confers an evolutionary advantage, with evidence suggesting that such abilities are present in animals including hermit crabs, octopus, and numerous species of fish (Sneddon et al., 2014). For this reason nociceptive pain has been considered 'adaptive' (Becker et al., 2018), in contrast to 'maladaptive' nociplastic and neuropathic forms of pain. Despite potential evolutionary advantage, the presence of pain is expected to exert significant negative impacts on an individual's quality of life (Wiseman-Orr et al., 2004; Botreau et al., 2007; Belshaw et al., 2015). Most ethical frameworks (Hernandez et al., 2018) would conclude that the relief of pain in their patients is an essential pursuit for veterinary surgeons. I have produced an example of an ethical matrix (Table 1) to summarise consequences of differing ethical approaches; the convergence of these ethical considerations strongly suggests that management of pain is ethically desirable. However, provision of suitable analgesia is predicated upon correct identification and assessment of pain, together with good evidence for chosen interventions.

Ethical framework	Provision of analgesia	Withholding of analgesia
Deontological: "*Ethical decisions are correct if they conform to a moral rule, law, or norm*".	Mandated by RCVS code of conduct to ensure welfare – pain negatively affects welfare therefore should be managed.	Would be acting against oath. Failure to prevent unnecessary suffering could lead to prosecution under the Animal Welfare Act, 2006.
Utilitarianism: "*greatest good (maximal pleasure, minimal suffering) for the greatest number of stakeholders*"	Relief of negative welfare associated with pain is likely to be considered a good outcome. In domesticated animals evolutionary advantages associated with pain are reduced/absent.	Cost and side effects of analgesia may impact this assessment, but are more likely to indicate that an alternative analgesic strategy be considered rather than no analgesia provided.
Virtue ethics: "*Virtues are character traits that are reliably present in individuals. Examples include compassion, discernment, trustworthiness, integrity, conscientiousness, initiative, self-discipline, responsibility, integrity and accountability."*	Compassion would promote the veterinary surgeon to take steps to alleviate pain.	Failing to treat pain in a sentient animal would be unlikely to be considered virtuous.
Contractarianism: "*Ethical rules, norms and obligations derive from an explicit or implied contract or mutual agreement."*	Animals are not considered able to consent to treatment or enter into contracts, this ethical approach may have limited utility in the question of analgesic provision.	
Ethics of care: "*recognises our relationships and obligations of care that follow from these*."	Analgesic provision is likely to be mandated by ethics of care.	Failure to provide analgesia is likely to be considered a breach of the ethics of care.

Table 1 Consideration of the ethical aspects of provision or withholding analgesia in domestic dogs and cats (after Hernandez et al., 2018)

1.1 Adaptive Pain

Although the current ISAP terminology does not define adaptive or maladaptive pain, acute pain is often considered to be an adaptive means of responding to potentially tissue damaging stimuli (Becker et al., 2018). As such, the components of adaptive pain comprise

nociceptive and inflammatory processes. Suprathreshold nociceptive stimulation provokes a reflex withdrawal reflex, increasing physical separation between the animal and the stimulus (Sherrington, 1910). Tissue damage causes the release and synthesis of signalling molecules (see peripheral sensitiation, below) which cause hyperalgesia and allodynia, manifesting in the behavioural consequences of protecting the injured site through alterations in posture or gait, and a reduction in movement based activities (Goldberg, 2017). The experience of pain impacts learning and memory, with the adaptive outcome of reducing further exposure to potentially damaging stimuli (Gerber et al., 2014). In previous surveys of the UK veterinary profession, a degree of post-operative pain was considered beneficial by approximately one-third of veterinary surgeons in preventing overactivity post-surgery (Capner et al., 1999). Recent guidelines for the delivery of anaesthesia to dogs and cats emphasize the requirement for effective control of perioperative pain (Grubb et al., 2020).

1.2 Nociception

Nociception is defined as "*The neural process of encoding noxious stimuli. Consequences of encoding may be autonomic (e.g. elevated blood pressure) or behavioural (motor withdrawal reflex or more complex nocifensive behaviour). Pain sensation is not necessarily implied*" (IASP, 2014), whilst nociceptive pain is defined as "*Pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors*" (IASP, 2014). Given the inability of veterinary patients to report their experiences, it has been proposed that nociceptive stimuli that would cause nociceptive pain in man are likely to

cause pain in other mammals; "*We can't always know that our patient does hurt, but we can do our best to ensure that it doesn't hurt"* (Mathews et al., 2014).

Nociception requires the *transduction* of physical stimuli (via thermo-, chemo-, or piezo-responsive ion channels, potentially acting in concert with specialised cutaneous schwann cells (Abdo et al., 2019), into alterations of membrane potential within nociceptor terminals of primary afferent fibres. The alterations in membrane potential may summate to generate action potentials that are *transmitted* via primary afferent Að- and C- nerve fibres to the dorsal horn of the spinal cord or trigeminal ganglion where they synapse; primarily in laminae I, II, and V, with second order neurons (which may generate reflex motor responses) and interneuron circuits. These laminae were described initially in the spinal cord of the cat (Rexed, 1952) but a homologous arrangement has been reported in macaques (Ralston, 1979) and rats (Lorenzo et al., 2008), with comparative organisation identified in elasmobranch fish (Cameron et al., 1990). At the synapses between primary afferent nociceptive fibres and second order neurones, the nociceptive signal may be subject to *modulation* by both local and descending pathways before the final output is *projected,* via neurons in ascending tracts (most importantly the spinocervicothalamic in domestic carnivores, as opposed to the spinothalamic tract in man, and spinoparabrachial tract in rodents (Dostrovsky and Craig, 2013)) of the spinal cord, to areas of the brain which co-ordinate nocifensive responses and have the capacity to generate perception of the stimulus as painful (Bell, 2018). Given the well preserved organisation and pharmacology of the nervous system related to nociceptive processing, it is considered that nociceptive processing is comparable between mammalian species (Sneddon, 2018), but the non-linear association between nociception and pain, and the translational validity of laboratory pain

models to clinical pain states has been suggested as a limitation to the development of novel analgesics for clinical use in man or animals (Mao, 2012).

1.3 Peripheral Sensitisation

Tissue damage leading to cell disruption and peripheral immune cell activation releases chemical mediators which potentiate local pain via i) direct activation of nociceptors (e.g. bradykinin, 5-hydroxytryptamine (5-HT), adenosine tri-phosphate (ATP), tumour necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and ii) sensitisation (less negative resting membrane potential and recruitment of 'silent' (high threshold) nociceptors) (e.g. bradykinin, prostaglandin E2 (PGE_2), 5-HT, protons (H^+), TNF-α, IL-1β, nerve growth factor (NGF), histamine) (Woolf et al., 2004). These changes are described as 'peripheral sensitisation', and are experienced as primary hyperalgesia ("*increased pain from a stimulus that normally provokes pain*"), and allodynia, ("*pain due to a stimulus that does not normally provoke pain*") (IASP, 2014). Peripheral sensitisation exhibits similar characteristics across mammalian species (e.g. Moncada et al., 1975; Xu et al., 2000), and the success of non-steroidal anti-inflammatory drugs to manage pain and inflammation in a range of species likely follows from these conserved mechanisms (Lees et al., 2004)

1.4 Central sensitisation

Central sensitisation is defined as "*Increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input*" (Arendt-Nielsen et al., 2018). The clinical consequences include secondary hyperalgesia (hyperalgesia beyond

the receptive field of the neurons which innervate the site of injury), and potentially allodynia. Central sensitisation has been documented following ovariohysterectomy surgery in dogs (Lascelles et al., 1997), and in dogs with coxofemoral osteoarthritis (Tomas et al., 2014). In man some biomarkers of central sensitisation are associated with reduced analgesic response to topical non-steroidal anti-inflammatory drug (NSAID) treatment (Edwards et al., 2016). Multiple mechanisms which may potentiate nociceptive signalling at the level of the spinal cord have been identified in experimental animal models and reviewed in detail (Sandkühler, 2009). These mechanisms include long term potentiation of nociceptive synapses, alterations in intrinsic plasticity, changes in inhibitory interneuron activity, altered balance between descending pro- and anti-nociceptive pathways, spreading of excitation from low threshold (Aβ) touch afferents to superficial (nociceptive related) synapses, and activation of microglia and astrocytes. Given that these examples of altered central nociceptive processing are associated with sensory phenomena of hyperalgesia and allodynia in animal models, it is believed that a number of these mechanisms are likely to be responsible for the development of hyperalgesia and allodynia in human clinical pain states (Jensen and Finnerup, 2014) and if this were to be the case, it would be reasonable to expect similar mechanisms to be responsible for central sensitisation in dogs and cats. Elucidating the relative contributions of differing mechanisms between different pain states and species would provide valuable insights as to potential targets for analgesics.

High intensity nociceptive input at the dorsal horn has the potential to alter the processing of subsequent nociceptive signalling (long term potentiation, LTP) via changes to glutamate-activated N-methyl-d-aspartate (NMDA) receptors, which result in loss of the magnesium

ion block of this ligand gated ion channel and augmented post-synaptic calcium ion influx in response to subsequent glutamate binding (Sandkühler, 2009).

Intrinsic plasticity relates to neuronal membrane excitability, and determines how a given membrane depolarization translates into firing of action potentials. Specific factors governing this property are not currently well described, but increases in excitability associated with experimental activation of metabotropic glutamate receptors or antagonism of tonic inhibition via $GABA_B$ receptors have been reported (Derjean et al., 2003).

Around 30% of neurons in the spinal dorsal horn are inhibitory interneurons; activity in these cells prevents spontaneous discharges in dorsal horn cells, suppresses excitatory impulses, reduces spreading of excitation to somatotopically inappropriate regions, suppresses excitation of nociceptive specific dorsal horn cells by cross-talk from touch sensitive Aβ-fibres, and reduces pre-synaptic excitatory neurotransmitter release and post-synaptic calcium dependent increases in membrane excitability (Sandkühler, 2009). Reduced activity in inhibitory interneurons may therefore contribute to perceptions of spontaneous pain, hyperalgesia, radiating pain, or allodynia.

Microglia and astrocytes, non-neuronal cells within the central nervous system, may contribute to hyperalgesia and allodynia, and function to maintain a pro-nociceptive state. They may be activated by spinal dorsal horn mediators glutamate, ATP, prostaglandins, and bradykinin resulting from peripheral nerve damage or inflammation. Production of factors such as TNFα, IL-1β, PGE_2 by non-neuronal cells can augment nociceptive synaptic function and membrane excitability (Milligan & Watkins, 2009).

Descending serotonergic and noradrenergic pathways from midbrain rostroventral medulla and locus coeruleus exert facilitatory and inhibitory effects on nociceptive synaptic

transmission (West et al., 2015). Injury and disease may alter the relative activation of these opposing systems resulting in a pro-nociceptive condition. These descending mechanisms have been explored in rats (Bannister et al., 2017), rabbits (Harris, 2016), and man (Pud et al., 2009), and are assumed present in all mammalian species, however prior to our work described later, investigations had not previously been performed in dogs.

1.5 Maladaptive Pain

Pain which occurs "*in the absence of ongoing noxious stimuli and does not promote healing and repair*" has been termed 'maladaptive' by clinicians in the pain field (Dickinson et al., 2010) and postulated to represent a disease process in itself (Tracey & Bushnell, 2009). In contrast other writers have argued that such forms of pain remain linked to evolutionary advantages for individuals (Walters, 2019). Regardless of whether this state is regarded as physiological or pathophysiological, ethical appraisal (Table 1) demands that in our domesticated animals, where 'maladaptive' pain has come to be separated from its potential evolutionary benefits, it should be managed effectively. The mechanisms of peripheral and central sensitisation summarised above may contribute to maladaptive pain within days of injury or, conversely, never at all, dependent on individual susceptibility (Edwards, 2005). Whilst NSAIDs have proven efficacy in the management of osteoarthritis associated pain in dogs (Sanderson et al., 2009), there is less evidence on appropriate strategies for management of cases which show inadequate response to NSAIDs (Moore, 2016), and in human medicine treatment of chronic pain remains challenging (Turk 2002).

1.6 Persistent Pain

The term 'persistent pain' is likely to be preferable to that of 'chronic pain' which, for human patients, is defined temporally as "*continuing or recurrent pain lasting for longer than 3 months*" (Treede et al., 2015). Persistent pain can be generated by continuing nociceptive or inflammatory pain, by neuropathic states, or by nociplastic changes within peripheral or central nervous systems; these may persist despite tissue healing sooner than 3 months, thus the temporal course of a painful condition cannot predict the underlying mechanisms which produce it. Clinical examples of persistent pain in dogs and cats are common and widespread, and include osteoarthritis, dental and periodontal lesions, aural disease, vertebral and spinal cord conditions, neoplasia, visceral pain, dermatological disease, and neuropathic conditions (Bell et al., 2014; Epstein, 2020; Menchetti et al., 2020; Monteiro 2020)

1.7 Welfare

Welfare is a multidimensional construct, encompassing physical and psychological dimensions. Broom (1986) defined this construct by writing "*the welfare of an individual is its state as regards its attempts to cope with its environment*". Initial approaches to promotion of welfare focussed on the minimising of negative states (hunger, thirst, discomfort, pain) and enabling the expression of normal behaviour (five-freedom model)[2]. Later thinking has highlighted the value of positive affective states in animals (Mellor, 2015) as a component in the provision of "*a good life*" for animals (as distinct from a "*life worth*

[2] https://webarchive.nationalarchives.gov.uk/20121010012427/http://www.fawc.org.uk/freedoms.htm accessed 16th September 2020

living", provided for by the five-freedom model) (Yeates, 2017). Assessment of welfare compromise over five domains (Mellor and Reid, 1994) has been modified to include considerations of positive affective states (Mellor and Beausoleil, 2015). Pain may impact welfare, and therefore quality of life (Broom, 2007), via both health related and affective domains (Mellor and Beausoleil, 2015) reflecting not only the sensory-discriminative aspects (location, intensity, character) of nociceptive stimuli (Botreau et al., 2007; Belshaw et al., 2015), but additionally their affective-motivational consequences (Auvray et al., 2010). Development of health related quality of life assessment tools has recently been undertaken in dogs (Reid et al., 2013). Such tools would be hoped to capture information relating to the impact of painful conditions in dogs, in relation to a more global appraisal of the dogs' welfare.

1.8 Summary

Nociception is an essential sense in free ranging animals, enabling the avoidance of harm related to suprathreshold nociceptive stimuli and in mammals, amongst other animals, associated affective components of nociception permit learning and the development of strategies to avoid similar stimuli in future. The physiology underlying nociception is comparable across mammalian species. Tissue injury induces an upregulation of nociception via peripheral and central sensitisation, with an evolutionary advantage in avoiding use of damaged tissues whilst healing occurs. Upregulation of nociception following healing, or in the absence of tissue injury is considered maladaptive, whilst ongoing disease processes such as osteoarthritis or neoplasia may result in persistent pain states. Both adaptive and

maladaptive pain have negative impact on welfare of domesticated animals and it is increasingly recognised that effective pain management is essential in the provision of humane care of animals.

2. Papers for consideration and accompanying commentary

2.1 Comparison of the efficacy of different opioids in managing surgical pain in canine patients.

As alluded to earlier, successful management of pain necessitates correct identification and assessment of pain, together with good evidence for chosen interventions.

Pain assessments incorporating behavioural indicators are more sensitive than those relying on physiological measurements in dogs (Holton et al., 1998a; Holton et al., 2001). The absence of pain may be therefore inferred by normal behaviour of the individual, however altered behaviour may not be specific for pain, particularly when effects of sedation, or anxiety resulting from unfamiliar environments and stimuli are considered (Short, 1998).

The evaluation of perioperative pain has been reported to be improved by interaction of the assessor with the animal and palpation of the surgical wound, compared to observation alone (Shih et al., 2008), therefore, if clinicians are in doubt as to the presence or absence of pain, palpation of potentially painful areas and encouraging the animal to move are likely to assist in diagnosis. Whilst assessments by experienced observers may reliably identify pain and estimate its magnitude, they are subject to significant inter-individual variability (Holton et al., 1998b). Given that a number of veterinary staff may treat and care for dogs whilst hospitalised, standardised clinical pain assessments should ideally minimise observer bias and furthermore define a pain score at which analgesic treatment is recommended. The

Glasgow Composite Measure Pain Scale was developed using psychometric principles to optimise validity, reliability, and responsiveness (Holton et al., 2001), and weighting of items in the scale described by Morton et al., (2005), providing a tool for research. A clinical tool was further developed from this, the short-form Glasgow Composite Measure Pain Scale (Reid et al., 2007), for which a suggested intervention pain score was determined (Reid et al., 2007). Use of this scale was subsequently validated in post-operative soft tissue and orthopaedic cases (Murrell et al., 2008), enabling clinical research to incorporate these scales as an outcome measure with a high degree of confidence that they will discriminate differing degrees of post-operative pain (Reid et al., 2018). Application the short form Glasgow Composite Pain Scale, was used to evaluate clinically relevant differences in analgesia induced by two opioid drugs in dogs in paper 1.

2.1.1 Paper 1. Hunt, J.R., Attenburrow, P.M., Slingsby, L.S., Murrell, J.C., 2013. Comparison of premedication with buprenorphine or methadone with meloxicam for postoperative analgesia in dogs undergoing orthopaedic surgery. Journal of Small Animal Practice 54, 418–424.

Prior to publication of this prospective, randomised positively controlled clinical study, few data were available to guide veterinary surgeons as to the relative efficacy of available opioid analgesics for the management of pain associated with orthopaedic surgery in dogs. Buprenorphine 0.007-0.02 mg kg^{-1} was considered to provide equivalent analgesia to morphine 0.3-0.8 mg kg^{-1} for a range of orthopaedic surgeries when assessed using a 4 point simple descriptive scale (SDS) to evaluate pain (Taylor & Houlton, 1984). Equivalent

analgesic efficacy was demonstrated between 0.01 mg kg $^{-1}$ buprenorphine and 0.3 mg kg $^{-1}$ morphine for dogs undergoing arthrotomy and assessed by a visual analogue scale (VAS) (Brodbelt et al., 1997). Both of the above studies were performed prior to validation of multidimensional acute pain scales in dogs, therefore data collected would have been subjective, and challenging to extrapolate to other populations. At the time that this research had been conducted the anaesthetics thiopental and halothane were commonly used, and these drugs were used in both of the above studies. By 2006 the most commonly used intravenous and volatile anaesthetic agents in dogs and cats were propofol and isoflurane (Brodbelt 2006). Recovery from thiopentone anaesthetic induction is significantly longer compared with recovery from propofol (Ko et al., 1999), whilst recovery from halothane anaesthesia has been reported to be significantly longer compared with isoflurane in dogs (Hellebrekers, 1986). Potentially, longer recovery times associated with thiopentone and halothane anaesthesia employed in these two studies may also have confounded the assessment of pain in the early postoperative period. Therefore, evaluation of the analgesics buprenorphine and methadone when used in combination with modern anaesthetic techniques and validated multidimensional pain scales was desirable to reflect current anaesthetic practice and fill the gap in the literature.

Buprenorphine has marketing authorisation for post-operative analgesia and sedation in dogs in the UK at a dose of 10-20µg kg^{-1}; and, at the time this study was conducted, was the most frequently prescribed opioid for premedication of dogs in the UK (Brodbelt, 2006), whilst in 2013 a racemic mixture of D- and L- methadone had recently received marketing authorisation for analgesia and premedication prior to general anaesthesia in dogs at doses of 0.5-1.0mg kg^{-1}.

Pharmacological differences between these two molecules suggested that there may be appreciable differences in efficacy for clinical pain. Levomethadone (L-methadone) functions as a full agonist (i.e. able to generate a maximal response) at the μ opioid receptor (Davis & Inturrisi, 1999), whilst buprenorphine's action is that of a partial agonist (i.e. unable to elicit a maximal response at full receptor occupancy) (Pick et al., 1997). Both drugs interact with additional receptor types. The dextrorotatory isomer of methadone (D-methadone) exhibits non-competitive NMDA receptor antagonism (Davis & Inturrisi, 1999), and methadone inhibits synaptic serotonin reuptake (Gillman, 2005). The contribution of NMDA antagonist activity of racemic methadone to anti-nociception is unclear (Carpenter et al., 2000), but it has been demonstrated to prevent morphine tolerance and NMDA-induced hyperalgesia (Davis & Inturrisi, 1999).

Buprenorphine functions as an agonist at the nociceptin/orphan FQ receptor (Lutfy & Cowan, 2004), and activation of this receptor by buprenorphine is considered to antagonise μ opioid receptor antinociception (Khroyan et al., 2009). Rodent nociceptive threshold testing models demonstrate biphasic (Pick et al., 1997) or bell shaped (Raffa & Ding, 2007) dose response curves associated with buprenorphine administration. It is postulated that these characteristics are the result of nociception/orphan FQ mediated attenuation of μ opioid anti-nociception (Khroyan et al., 2009).

The clinical relevance of the properties of buprenorphine are not fully understood. In a study of dogs undergoing ovariohysterectomy, no difference in VAS pain scores was identified in dogs treated with 40μg kg^{-1} buprenorphine compared with 20μg kg^{-1} (Slingsby et al., 2011); suggesting that buprenorphine associated analgesia had reached a plateau at the 20 μg kg^{-1} dose, but that analgesia was not antagonised at the higher dose. However,

antagonism of sufentanil (a potent μ opioid agonist) antinociception by buprenorphine has been demonstrated in dogs undergoing ovariohysterectomy (Goyenechea Jaramillo et al., 2006).

Administration of methadone (0.3 mg kg^{-1}) by intravenous and extradural routes in dogs undergoing surgical repair of cruciate ligaments (Leibetseder et al., 2006) demonstrated that both intravenous methadone and extradural methadone provided adequate analgesia for approximately six hours in this study. In that study, extradural administration of methadone significantly reduced the concentration of isoflurane required to maintain anaesthesia, compared to intravenous methadone. This suggests that the intraoperative anti-nociception associated with extradural administration was more profound than that elicited by intravenous methadone.

Given the differences between the two drugs, we designed the study to rigorously compare, using validated pain scoring tools, the analgesic effects of either 20μg kg^{-1} buprenorphine (group B) or 0.5mg kg^{-1} methadone (group M) intramuscularly at the time of premedication, in conjunction with meloxicam administered subcutaneously following induction of anaesthesia. Pain was assessed using the Short Form Glasgow Composite Pain Scale (GCPS-SF) and dynamic interactive visual analogue scale (DIVAS). The results of the study demonstrated clinically relevant differences in post-operative pain scores between dogs treated with the two analgesics. Over the course of the observation period mean pain scores were higher in group B compared to group M as assessed by both DIVAS and GCPS-SF. At time points 210, 240, 360 and 480 minutes post premedication, mean GCPS-SF was significantly higher in group B compared to group M. The differences in pain scores had real-world consequences, in that a significantly greater proportion of dogs that were treated

with buprenorphine required additional analgesia prior to scheduled post-operative analgesics, compared with dogs treated with methadone. This work also demonstrated that significant numbers of dogs undergoing orthopaedic surgery and treated with systemic analgesics were likely to require additional analgesia prior to scheduled dosing, underscoring the importance of assessing pain in individual patients and considering alternate analgesic strategies, such as locoregional techniques (Bini et al., 2018).

Subsequent work has demonstrated similarly reduced pain scores and requirement for additional analgesics in both dogs (Shah et al., 2018b) and cats (Shah et al., 2018a) undergoing ovariohysterectomy treated with methadone compared with buprenorphine, although smaller studies in mixed sex cats undergoing castration or ovariohysterectomy have not identified such differences (Bortolami et al., 2013, Slingsby et al., 2015). A possible explanation for this discrepancy is the inclusion, in the latter two studies, of both castration and ovariohysterectomy surgeries, thus increasing variability of the associated surgical pain (Quarterone et al., 2017); and the evaluation of three opioid groups (methadone, buprenorphine, and butorphanol), thereby reducing the power of the studies to detect differences.

The statistical handling of Glasgow Composite Pain Scale (GCPS-SF) data employed in paper 1 relied on transforming the recorded whole number scores to a fraction, in order to account for the application of different denominator values in dogs which were ambulatory compared with those which were not. This approach enabled the longitudinal evaluation of dogs from the early (non-ambulatory) post-operative period to the study conclusion 8 hours

post pre-medication. However, this transformation has been criticised, as conversion to a fraction may alter the measurement properties of the scale (Reid, 2019; personal communication). An alternative proposed approach would be to score any dogs that remain too heavily sedated to move as maximal in the movement category (Reid, 2019; personal communication) though this may over-estimate pain scores. A possible consequence of this approach may be the overuse of analgesics, which may be associated with vomiting or a delayed return to eating (Bini et al., 2018).

Future studies which evaluate agreement between these two different approaches of processing GCPS-SF scores, compared with a dynamic interactive visual analogue scale completed by the same observer, might provide further evidence as to the most appropriate means of handling GCPS-SF data in these circumstances.

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PAPER

Comparison of premedication with buprenorphine or methadone with meloxicam for postoperative analgesia in dogs undergoing orthopaedic surgery

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Objectives: To determine whether methadone, administered before orthopaedic surgery, results in improved postoperative analgesia compared to buprenorphine.

Methods: Thirty-eight dogs undergoing orthopaedic surgeries (the majority being tibial tuberosity advancement or elbow arthrotomy) were premedicated with 0·03 mg/kg acepromazine and either 20 µg/kg buprenorphine or 0·5 mg/kg methadone, intramuscularly, allocated randomly. Anaesthesia was induced with propofol intravenously to effect and maintained with isoflurane in oxygen. 0·2 mg/kg meloxicam was administered at anaesthetic induction. Sedation was assessed by means of a dynamic interactive visual analogue and simple descriptive scales and pain by dynamic interactive visual analogue and the short form Glasgow composite pain scales, by a single observer blinded to treatment group at intervals for 8 hours following premedication.

Results: Sedation scores were higher than baseline in both groups following premedication until the end of the assessment period (P=0·0001), with no differences between groups. Pain scores were lower overall in dogs premedicated with methadone (dynamic interactive visual analogue scale P=0·048; short form Glasgow composite pain scale P=0·0045), and these dogs required less additional analgesia (42%, compared to 79% premedicated with buprenorphine, P=0·045).

Clinical Significance: At the doses investigated, methadone produced superior analgesia to buprenorphine for 8 hours postoperatively in dogs undergoing orthopaedic surgery.

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INTRODUCTION

The majority of opioids used as analgesics display agonist or partial agonist activity at µ receptors. Mu receptor agonists are ligands which, when applied to the µ receptor in an appropriate concentration to saturate the receptor-binding sites, can produce the maximal pharmacodynamic response and are regarded as highly efficacious analgesics (Tranquilli *et al.* 2007). Partial µ agonists also activate the µ receptor, however, the resulting pharmacodynamic response is submaximal, even at full saturation of the receptor-binding sites (Tranquilli *et al.* 2007). Therefore partial µ agonists are considered to be less efficacious analgesics than full µ agonist drugs (Zuurmond *et al.* 2002).

Buprenorphine, a partial µ opioid agonist (Lutfy & Cowan 2004) is licensed for postoperative analgesia and sedation in dogs

in the UK and, in the most recent surveys of the profession, was the most commonly used opioid for premedication of dogs in the UK (Brodbelt 2006) and in South Africa (Joubert 2006). Buprenorphine is characterized as a partial agonist at the μ opioid receptor, antagonist at the κ opioid receptor (Pick *et al.* 1997) and agonist at the nociceptin/orphan FQ receptor (Lutfy & Cowan 2004), and is reported to have a biphasic (Pick *et al.* 1997) or bell-shaped (Raffa & Ding 2007) dose-response curve in terms of antinociception in rodent models. This has been attributed to an agonist action at the nociceptin/orphan FQ receptor attenuating μ opioid receptor-mediated antinociception (Khroyan *et al.* 2009). However, a clinical study in dogs suggested that the dose-response curve for buprenorphine reached a plateau at the 20 μg/kg, but that no antanalgesia developed at higher doses (Slingsby *et al.* 2011). There are a small number of published studies on the efficacy of buprenorphine in providing analgesia for orthopaedic surgery in dogs. These studies suggest that buprenorphine administered in a dose range of 0·007 to 0·02 mg/kg provides equivalent analgesia to morphine at doses between 0·3 and 0·8 mg/kg for a range of orthopaedic surgeries (Taylor & Houlton 1984, Brodbelt *et al.* 1997).

A racemic mixture of methadone has recently been licensed for analgesia and premedication before general anaesthesia in dogs. l-methadone is characterized as a full agonist at the μ opioid receptor, whilst the d-isomer exhibits non-competitive N- methyl D- aspartate (NMDA) receptor antagonism (Davis & Inturrisi 1999) and methadone is also reported to act as a weak serotonin reuptake inhibitor (Gillman 2005). Whilst the contribution of NMDA antagonist activity to antinociception is unclear (Carpenter *et al.* 2000), it has been linked to the prevention of morphine tolerance and hyperalgesia (Davis & Inturrisi 1999), and therefore racemic methadone has the potential to contribute to provision of multi-modal analgesia. Methadone has been administered by intravenous (iv) and extradural routes to produce analgesia in dogs undergoing surgical repair of cruciate ligaments (Leibetseder *et al.* 2006), and iv was considered to provide adequate analgesia for approximately 6 hours.

Because of the described pharmacological differences between methadone and buprenorphine, methadone may provide more efficacious analgesia compared to buprenorphine when administered to dogs undergoing moderately to severely painful surgeries (Cowan *et al.* 1977). However, this supposition has not been formally investigated in a robust clinical study in dogs. The aim of this study was to compare pain and sedation in dogs undergoing orthopaedic surgery administered either methadone or buprenorphine for premedication and repeated once in the postoperative period. It was hypothesized that premedication with methadone would produce greater antinociceptive efficacy during surgery, and analgesia after surgery, than premedication with buprenorphine, manifested by a lower requirement for volatile agents intraoperatively and lower pain scores and fewer additional analgesic interventions postoperatively in dogs, with no significant differences in sedation between treatments. The ultimate aim of the study was to provide clinically relevant and robust data to assist clinicians in decision-making around opioid selection in dogs undergoing moderately to severely painful surgery.

MATERIALS AND METHODS

An observer blinded, randomized (random number generator; www.random.org) prospective clinical study was conducted. The study was approved by local ethical review UIN UB/12/015 and by the Veterinary Medicines Directorate ATC-S 031.

Thirty-eight client-owned dogs aged six months or more and American Society of Anesthesiologists (ASA) 1 or 2 on preanaesthetic examination, presented for orthopaedic surgery at St. David's Veterinary Hospital, Exeter were recruited with written, informed owner consent. Dogs were excluded if they were known to be hypersensitive to any of the study products, if they had any gastrointestinal disease that would preclude the administration of non-steroidal anti-inflammatory drugs (NSAIDs), or if they were receiving opioid analgesics before recruitment to the study (e.g. on the day before surgery).

Calculation of sample size with Altman's nomogram, employing an α of 0·05 and β of 0·8 predicted that 15 animals per group were required to detect a difference between isoflurane settings of 2·25 *versus* 2% (using an sd of 0·23 as determined by pilot data) (Petrie & Watson 2006).

Dogs were fasted for a minimum of 8 hours before undergoing anaesthesia, but were provided with water until the time of premedication. Following admission, dogs were weighed (Burtons Medical Equipment Ltd), housed in stainless steel kennels and provided with suitable soft bedding. Baseline measurements were collected for heart rate (HR), respiratory rate (RR), body temperature and mechanical nociceptive threshold (MNT) at the proposed site of surgery. Baseline assessments of sedation and pain were also performed. Measurements and assessments were always performed by the same observer (JRH) who was blinded to treatment group.

Premedication (timepoint P), consisting of 0·02 mg/kg acepromazine (ACP 2 mg/mL; Novartis Animal Health), and either 20 μg/kg buprenorphine (Vetergesic Solution for Injection; Alstoe Animal Health) (group B) or 0·5 mg/kg methadone (Comfortan®; Dechra Veterinary Products Ltd) (group M), mixed in the same syringe, was administered into the cervical epaxial muscles. Thirty minutes later anaesthesia was induced by iv injection of propofol into a cephalic vein until jaw relaxation and suppression of pharyngeal reflexes was adequate for orotracheal intubation with a cuffed endotracheal tube (ET). The volume of propofol required was recorded. Anaesthesia was maintained with isoflurane vapourized in oxygen, delivered via an appropriate non-rebreathing system. Meloxicam (Metacam injection for dogs and cats™; Boehringer-Ingelheim) and amoxicillin-clavulanate (Synulox injection™; Pfizer) at standard doses were administered subcutaneously following induction of anaesthesia.

Monitoring of pulse rate and RR was performed by palpation and observation following induction of anaesthesia. Continuous monitoring of HR, RR, haemoglobin saturation with oxygen (SpO_2) and end tidal carbon dioxide partial pressure ($P_E'CO_2$) was performed during surgery (Patient Monitor G3C; Meditech Inc). Recordings of these parameters and vapourizer setting were made every 5 minutes throughout the duration of anaesthesia.

During surgery a registered veterinary nurse determined management of anaesthesia. Increases in values of heart or RR of greater than 25% relative to values recorded before the start of surgery, after induction of anaesthesia and transition to isoflurane anaesthesia, in response to noxious stimulation, were treated by increasing the dialled concentration of isoflurane by 0·25 to 0·5%, as per routine practice in the clinic where the study was conducted. Following surgery and postoperative radiographs and dressings, isoflurane administration was stopped and extubation performed according to routine clinical practice. Time from discontinuation of isoflurane to headlift, sternal recumbancy and standing were recorded, and the quality of recovery from anaesthesia was judged, from 0 to 3, using a simple descriptive scale (SDS) (Table 1).

Measurements and assessments of physiological parameters, pain and sedation were performed postoperatively at 30, 60, 90, 120, 150, 180, 210, 240, 300, 360 and 480 minutes after administration of premedication, unless the animal was still anaesthetized.

Sedation was assessed by observing the dog in its cage, approaching the cage and calling the dog, then opening the cage, stroking the dog, and encouraging it to stand and walk. A dynamic interactive visual analogue scale (DIVAS) score (as described by Slingsby & Waterman-Pearson (2000)) for sedation was assigned by the assessor, on a 100-mm scale where 0 indicates no sedation and 100 indicates completely sedated and unrousable, and then a score from 0 to 3 was assigned using an SDS (Table 2).

Pain was assessed by observing the dog in its cage, approaching the cage and opening the door, encouraging the dog to stand and walk if capable, and by applying gentle palpation using the fingers of one hand approximately 1 inch from the wound. A pain DIVAS score was determined (0-100; where 0 indicated no pain and 100 the worst pain imaginable) according to the assessors' interpretation of the dogs' responses to these interactions, and then a score for pain level was assigned using the short form Glasgow composite pain score (SF-GCPS) (available from http://www.gla.ac.uk/media/media_233876_en.pdf). If a DIVAS score greater than 40 mm was assigned, or SF-GCPS was greater than 5 of 20 (if the dog was non-ambulatory) or 6 of 24 (if the dog could walk) additional analgesia was provided with methadone 0·5 mg/kg intramuscularly and pain reassessed 30 minutes later. If pain scores remained above the level for threshold or requirement for additional analgesia was considered to be clinically indicated a second dose of 0·5 mg/kg methadone was administered and pain reassessed after 30 minutes. No dogs required further analgesia following a second administration of methadone, but had additional analgesics been necessary the assessor would have implemented an analgesic strategy appropriate for the individual concerned using non-opioid analgesics. Clinical pain assessment was performed before measurement of MNT at each time point.

MNT was measured using a handheld force-meter (PROD; Topcat Metrology) equipped with a 2-mm probe tip, with which force was applied at a rate of 2 N per second until the dog showed signs of wanting to avoid the stimulus (moving away, lifting a leg, assuming submissive posture) or of being in pain (crying, snapping). If a 20 N force was exerted without the dog responding, the mechanical threshold measurement was stopped and a value of 20 N recorded. To obtain a baseline MNT, three measurements were made at the site of proposed surgery 5 to 10 minutes apart, and the mean used as the baseline. At all other time points MNT was measured once.

Following assessment at time point P+300 minutes, dogs that had not previously been administered rescue analgesia were treated with a second opioid treatment according to their group (B or M), equivalent to the original premedicant dose of the allocated opioid treatment. In dogs that had received rescue analgesia, assessments were continued according to the study protocol but no further analgesia was given at P+300 minutes, unless deemed necessary based on pain assessment.

Table 1. SDS scheme used to attribute scores for quality of recovery from anaesthesia

SDS	Quality of recovery
0	Poor (dog shows major signs of excitement during recovery to sternal recumbency such as thrashing in cage or moving around rapidly unaware of surroundings, growling; which does not respond to gentle handling)
1	Moderate (dog shows some signs of excitement during recovery to sternal recumbency such as thrashing in cage or moving around rapidly unaware of surroundings, growling; but responds to gentle handling by calming down)
2	Good (mild signs of excitement which resolves quickly and dog becomes calm)
3	Excellent (dog is calm and relaxed during recovery)

Table 2. SDS scheme used to attribute scores for level of sedation

SDS	Behaviour indicative of sedation
0	None
1	Mild (dog was relaxed, but could be roused and could walk with little or no ataxia)
2	Moderate (dog was in sternal or lateral recumbency, but could be roused and had obvious signs of ataxia)
3	No response to stimulation

Statistical methods

Data were assessed for normality and appropriate statistical techniques for parametric and nonparametric data applied (Prism 5 for Mac OSX; GraphPad Software, Inc). The SF-GCPS was converted to a decimal number, the denominator determined by whether the total possible maximum score was 24 (if the dog was ambulatory) or 20 (if the dog could not walk). In this way, the threshold for intervention became 0·25, being a score of 5 out of a possible 20, or 6 out of a possible 24. Means of parametric data such as age, body weight, baseline HR, RR, MNT, surgery time, and time to headlift, sternal and standing from isoflurane discontinuation were compared with Student's *t* test and are presented as mean ±sd. The Mann-Whitney U test was used to assess nonparametric data such as baseline SDS pain and sedation, baseline SF-GCPS, recovery quality and number of rescue analgesic interventions administered per animal. Mixed between-within group

ANOVA was used to evaluate HR, RR, isoflurane vapourizer setting, $PE'CO_2$, SpO_2, DIVAS pain and sedation, GCPS and MNT over time. P values ≤ 0·05 were considered significant. Where multiple comparisons were performed the Bonferroni correction α/n was used to calculate relevant alpha; data were analysed at nine time points, therefore P values ≤ 0·006 were considered significant. For repeated measures analysis if one data point for an individual was missing it was replaced with the next subsequent value. If more than one data point was missing the individual was excluded from analysis. The proportion of dogs in each treatment group requiring rescue analgesia was compared with a Fisher's exact test.

RESULTS

Demographical data

There were no differences between groups with respect to age (overall mean 59 ±33 months), weight (27 ±13 kg), sex and neutering status and breed distribution. Males comprised 60% of the population in both groups. The most represented breeds were Labrador retrievers (n=6), Staffordshire bull terriers (n=5), Border collies (n=5), springer spaniels (n=4), cocker spaniels (n=2), golden retrievers (n=2) and Jack Russell terriers (n=2).

There were no differences in the types of surgery performed in the two groups (P=0·24). The most common surgeries performed were tibial tuberosity advancement (n=19) and elbow arthrotomy (n=8).

Pre- and Intraoperative assessments

Baseline HRs [overall mean 114 ±30 beats per minute (bpm)] and RRs (32 ±9 breaths per minute (brpm) did not differ between groups, although 50% of dogs in both groups were panting at baseline. Following premedication, HRs were significantly lower than baseline (P<0·0001) (M 85 ±24; B 81 ±28 bpm) with no difference between groups. RRs were unaltered from baseline, 50 to 60% of dogs remained panting in each group.

There were no differences in pain between groups at baseline or following premedication (Figs 3 and 4) and pain scores did not change following premedication.

After premedication, sedation was not different between groups but was increased significantly in both groups compared to baseline [DIVAS 34 ±20; SDS 2 (1-3) P<0·0001]. MNTs were not different between groups but were significantly higher following premedication than baseline values (9·6 ±4 N (baseline) 14 ±5N (after premedication), P<0·0001).

The dose of propofol required for induction of general anaesthesia was significantly lower in group M compared with group B (M 3·7 ±0·8; B 4·3 ±0·7 mg/kg, P=0·009). Because of differences in the duration of anaesthesia between individual dogs isoflurane vapourizer settings were only compared for the first 45 minutes of anaesthesia to optimize the number of usable datasets, however, there was no significant difference between groups (mean vapourizer setting 2·2 ±0·18%). There were no differences between groups with respect to intraoperative HR, RR, $PE'CO_2$ or SpO_2.

Duration of anaesthesia was slightly longer in group B (M 58 ±10; B 69 ±17 minutes, P=0·021) but there was no difference between groups with regard to duration of surgery (34 ±11 minutes).

There were no differences between groups with regard to time to headlift (M 23 ±15; B 20 ±18 minutes), sternal (M 52 ±39; B 33 ±23 minutes) or standing (M 93 ±76; B 127 ±84 minutes), nor with regard to recovery quality [M 2 (1-3); B 2 (1-3)].

Postoperative assessments

Physiological variables

No differences were detected between groups with respect to heart and RRs postoperatively. Heart and RRs remained significantly lower than baseline, but within physiological limits, from P+150 minutes until the end of the assessment period, and were not different between groups.

Postoperative sedation

Sedation scores (DIVAS and SDS) remained elevated compared to baseline from P+150 minutes until the end of the observation period (P<0·0001) in both groups, with no statistically significant differences between groups (Figs 1 and 2).

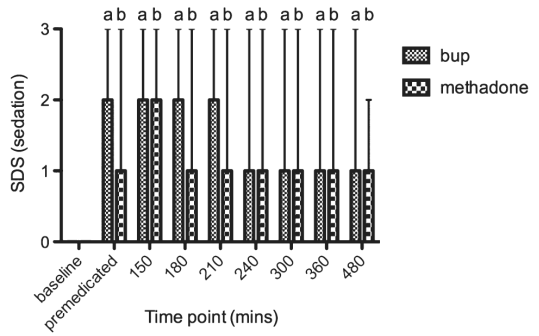

FIG 1. Median SDS sedation scores over time, error bars indicate range. "a" Denotes significant difference to baseline and sedated time points in group B (n=17), "b" denotes significant difference to baseline and sedated time points in group M (n=19)

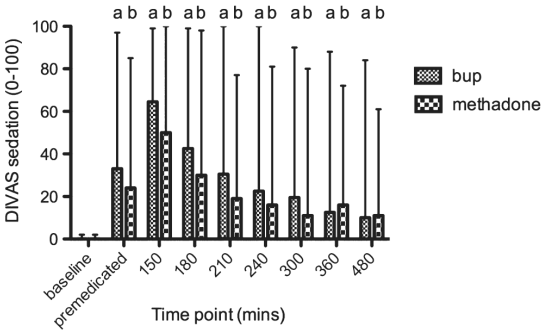

FIG 2. Mean DIVAS sedation scores over time, error bars indicate sd. "a" Denotes significant difference to baseline and sedated in group B (n=16), "b" denotes significant difference to baseline and sedated in group M (n=19)

Postoperative pain

Pain scores were significantly higher in group B overall as assessed by DIVAS (P=0·048) and but were not different at any single time point (Fig 3). Pain scores assessed by SF-GCPS were higher overall in group B (P=0·0045) and higher at time points 210, 240, 360 and 480 minutes (Fig 4). Within each group, pain scores were higher at all time points postoperatively compared to baseline and sedated time points (P<0·0001).

Intervention analgesia

Eight of 19 dogs (42%) in group M and significantly more [15 of 19 (79%), P=0·045] in group B required additional analgesia before the planned administration of second dose of the test analgesic (buprenorphine or methadone) at P+300 minutes. Within the total observation period (up to P+480 minutes), no animals required further analgesic following this intervention.

Of the animals that required additional analgesia; dogs in groups M and B required a mean of 1·4 and 1·6 administrations of methadone, respectively, which was not significantly different between groups.

Survival curves, using requirement for intervention analgesia, were plotted to compare the adequacy of analgesia throughout the study period between groups (Fig 5). The two curves were significantly different (P=0·029). Median time to additional analgesia in dogs premedicated with buprenorphine was 180 minutes. It was not possible to calculate this for the methadone group as fewer than 50% of dogs required rescue analgesia.

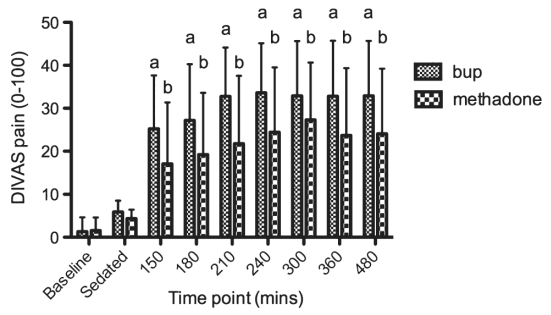

FIG 3. Mean DIVAS pain scores over time, error bars indicate sd. "a" Denotes significant difference to baseline and sedated in group B (n=17), "b" denotes significant difference to baseline and sedated in group M (n=19)

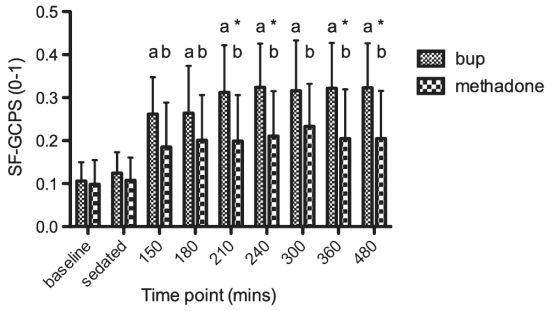

FIG 4. Mean SF-GCPS pain scores over time, error bars indicate sd. "a" Denotes significant difference to baseline and sedated in group B (n=17), "b" denotes significant difference to baseline and sedated in group M (n=19). * denotes a significant difference between groups (P<0·006)

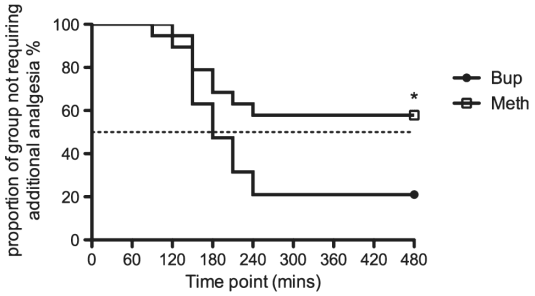

FIG 5. Kaplan–Meier survival curve, illustrating survival as no requirement for additional analgesia. * Denotes significant difference between two curves (P=0·029). The dotted line indicates 50% of the population, and intersects the survival curve at the median analgesia duration from time of premedication

Mechanical Nociceptive Threshold

Postoperatively, padded dressings were applied to wound areas, and it proved impossible to meaningfully relate postoperative MNTs to preoperative values, therefore these data are not presented here.

DISCUSSION

Dogs administered 0·5 mg/kg methadone and meloxicam had significantly lower postoperative pain scores and significantly lower requirement for intervention analgesia compared to those administered 0·02 mg/kg buprenorphine and meloxicam. As such, this clearly demonstrates the analgesic benefit of methadone for provision of analgesia in this acute surgical pain model. The doses of buprenorphine and methadone were within the licensed dose range for each drug and reflect current clinical practice. There are no published reports of the relative efficacies of methadone and buprenorphine in dogs, however, relative analgesic potencies of opioid analgesics in man, compared to morphine, are reported to be 1 for methadone and 25 for buprenorphine (Inturrisi 2002), therefore the doses used in the study should have been equianalgesic. Regardless of the relative efficacies of the drugs in nociceptive models, the aim of this clinical study was to compare the two opioids at their licensed dose rates in order to provide information to veterinary surgeons on using the products according to the datasheet indications. The finding of greater analgesic efficacy associated with administration of methadone suggests either a "ceiling" effect to the μ opioid receptor-mediated analgesic effect of buprenorphine, or augmentation of the opioid analgesic effect of methadone via non-opioid mechanisms. This finding may relate to the classification of methadone as a full, and buprenorphine as a partial, μ opioid receptor agonist, however, the concept of full and partial agonist responses also needs to

be considered in light of the stimulus challenge (Raffa & Ding 2007). It is believed that the lower intrinsic efficacy of buprenorphine compared to, for example, morphine, limits antinociceptive effects in nociceptive test situations, however, with increasing magnitude of nociceptive stimuli there is some experimental evidence that morphine also begins to function as a partial agonist and maximal efficacy is reduced, whilst that of the potent agonist sufentanil remains unchanged (Dirig & Yaksh 1995). A large amount of experimental work regarding opioid agonist analgesia has been performed using a model of noxious heat, therefore it has been suggested that differences in antinociceptive activity of different opioids is not a function of the relative intrinsic efficacy of an agonist, but rather reflects the differential activation of A-delta fibre mechanothermal nociceptors at high rates of heating compared to stimulation of C fibre polymodal nociceptors at lower rates of heating, and that differences in the effectiveness of different opioid drugs to attenuate A-delta fibre mediated nociception may be responsible for the reported change in maximum efficacy in thermal nociceptive models (McCormack *et al.* 1998).

Sedation may confound assessments of pain (Lasagna 1960, Hunt *et al.* 2013). However, in this study the finding of equivalent levels of sedation in both groups throughout the period of study suggests that the pain scores were unlikely to have been confounded by sedation.

This data contrast with the results of previous studies that found equivalent analgesia from buprenorphine (0·006 and 0·007-0·02 mg/kg) and morphine (0·2 and 0·3-0·8 mg/kg) in dogs undergoing orthopaedic surgery (Taylor & Houlton 1984, Brodbelt *et al.* 1997). Both morphine and methadone are full μ opioid agonists (Adams *et al.* 1990), therefore the disparity in findings may reflect differences in the methodology of this study to earlier work. Taylor and Houlton (1984) utilized an SDS to assess analgesia, which would be expected to be less sensitive than the SF-GCPS and DIVAS used in this study. Brodbelt *et al.* (1997) assessed pain by means of a DIVAS, however, the surgical procedures were performed by four different surgeons, which may have increased variability in pain scores between animals. In this study, all the surgeries were performed by the same surgeon and the assessments were made by a single observer. There were also no differences between groups with respect to surgery duration or type and dressings were applied to all dogs after surgery. The attention to standardization of these factors may have increased the power of this study to determine differences in analgesia provided by methadone and buprenorphine in the postoperative period. As this study employed higher doses of buprenorphine and full μ agonist, it may be that differences in analgesic efficacy between buprenorphine and full μ agonists become more apparent at higher doses than those used in earlier studies.

Although recorded anaesthesia times were slightly longer in buprenorphine-treated dogs, the surgery times between groups were not different, suggesting that the level of invasiveness and trauma caused by the surgeries in both groups would be expected to be similar, therefore we do not consider that the difference in anaesthesia times would have had a significant influence on our findings.

The study population underwent major surgery without the concurrent use of peripheral or neuraxial local anaesthetic techniques, reflecting current practice in many first opinion and secondary veterinary referral centres. Significantly fewer dogs in the methadone group required additional analgesia compared with dogs that received buprenorphine. However, intervention analgesia was required in some dogs in both groups, highlighting the importance of regular pain assessment, irrespective of analgesic drug regimen. Within the cohort of dogs that required intervention analgesia, there was no difference in the dose requirement for methadone, irrespective of premedication opioid. This suggested that residual occupancy of μ receptor sites by buprenorphine did not impair the efficacy of the subsequently administered full μ agonist, which is in contrast to the findings of other studies (Goyenechea Jaramillo *et al.* 2006). Recent clinical evidence in humans has shown that buprenorphine does not antagonize morphine analgesia (Oifa *et al.* 2009), and the results presented here suggest that buprenorphine premedication should not dissuade clinicians from subsequently administering full μ agonists, if indicated, on the basis of pain assessment. All the dogs that received intervention analgesia required treatment within the expected duration of action of each drug (buprenorphine reported to have a duration of 6 (Shih *et al.* 2008) to 16 hours (Ko *et al.* 2011), methadone reported to be 6 hours (Leibetseder *et al.* 2006)), again highlighting the importance of regular pain assessment postoperatively. Both drugs were expected to produce analgesia of sufficient duration that dogs were comfortable until the repeat analgesic administration at 5 hours. The difference in requirement for rescue analgesics is therefore considered related to a difference in efficacy between methadone and buprenorphine, rather than due to pharmacokinetic differences between the two drugs.

It was not possible to demonstrate postoperative secondary hyperalgesia by assessment of MNT in dogs in either group in this study, probably a consequence of inaccurate measurements obtained because of the presence of the padded dressings. Values of MNT increased following premedication, which may reflect antinociception as a result of the opioid component of premedication, however, increases in MNT have been observed in cats treated with acepromazine alone (Steagall *et al.* 2008). Differentiating an antinociceptive effect from a sedative effect in α_2 agonist-treated animals has been described using electrophysiological measurements in dogs (van Oostrom *et al.* 2011) but this methodology is inappropriate for a clinical study. Secondary hyperalgesia is commonly reported after surgery in dogs, even when NSAIDs and opioids are administered for analgesia (Slingsby & Waterman-Pearson 2001), reflecting altered sensory processing following tissue injury. It was hoped that the antagonist action of methadone at the NMDA receptor would manifest clinically as a reduction in secondary hyperalgesia. However, as this was a clinical study altering the postoperative care by removing the dressings was considered undesirable and therefore not attempted.

Methadone decreased the dose of propofol required to induce general anaesthesia compared to buprenorphine; veterinary surgeons should be aware that dogs premedicated with methadone as opposed to buprenorphine may require less iv induction agent.

Despite this induction agent sparing effect, relative to buprenorphine, there were no differences between groups with respect to isoflurane vapourizer settings. Similar to many veterinary centres within the UK that do not have access to a specialist veterinary anaesthetist, the mainstay of intraoperative analgesia is by inhalation of volatile agents, therefore vapourizer settings were considered as a proxy measure of intraoperative antinociception. The fact that there was no difference between groups may reflect the fact that relying solely on either methadone or buprenorphine premedication for intraoperative analgesia will not abolish nociceptive responses and specific intraoperative analgesics (e.g. systemic opioids or use of neuraxial or regional analgesia techniques) should be considered in addition to opioid premedicants.

In conclusion, lower pain scores and a reduced need for additional analgesics can be expected in dogs undergoing orthopaedic surgery treated with methadone and an NSAID compared to those treated with buprenorphine and an NSAID. The results of this study indicate that methadone premedication should be considered for dogs undergoing orthopaedic surgery and that methadone may be used as an intervention analgesic for animals experiencing pain following orthopaedic surgery, even if they were premedicated with buprenorphine. Pain assessments should be performed at least every 30 to 60 minutes following surgery when systemic analgesics are used as the sole means of providing analgesia.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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2.2 Defining the Prescribing of Analgesics in Clinical Practice

Given clear evidence that methadone acts as a more efficacious analgesic, at authorised doses, compared with buprenorphine in dogs, it was important to document the analgesic strategies currently used by veterinary surgeons in the UK and identify areas where education or training could benefit animal welfare. The provision of analgesia to dogs and cats under veterinary care has historically been inadequate (Capner et al., 1999; Lascelles et al., 1999), and failed to meet client expectations (Demetriou et al., 2009). However increased availability of analgesics with marketing authorisation for use in dogs and cats and wider access to continued professional development on the subject of analgesia may be expected to improve prescribing rates in the 17 years since the previous studies were performed. Evidence from other countries had documented increasing levels of perioperative analgesic prescription over time to cats and dogs by veterinary surgeons (Joubert, 2006; Hewson et al., 2006); alongside some information suggesting improved analgesic treatment of cats undergoing neutering surgery from the UK, together with Australia and New Zealand (Farnworth et al., 2014). Previous work had also demonstrated reduced prescription of analgesics to cats compared with dogs; and higher analgesic prescription rates amongst female veterinary surgeons and veterinary surgeons who had graduated more recently (Capner et al., 1999; Lascelles et al., 1999). It was therefore important that a comprehensive survey of the profession, which could be related to the earlier findings of Capner et al. (1999) and Lascelles et al. (1999), was undertaken in the UK to enable benchmarking of the current level of analgesic prescriptions.

2.2.1 Paper 2. Hunt, J.R., Knowles, T.G., Lascelles, B.D.X., Murrell, J.C., 2015. Prescription of perioperative analgesics by UK small animal veterinary surgeons in 2013. Veterinary Record 176, 493.

In order to determine whether provision of analgesia overall to cats and dogs had increased in the UK since 1999, a cross-sectional survey was designed and distributed. The primary research question was whether provision of perioperative analgesia to cats and dogs overall had increased in the UK since the previous survey in 1999. Secondary research questions included whether use of multi-modal analgesic strategies had increased compared with previous findings, whether a discrepancy remained between the prescription of analgesics for cats compared with dogs, and whether levels of analgesic prescription continued to be influenced by gender and duration of qualification of the veterinary surgeon.

Printed copies of the survey were distributed, along with postage paid envelopes, to four thousand randomly selected veterinary surgeons engaged in small animal practice (approximately 20% of the total number of veterinary surgeons registered with the Royal College of Veterinary Surgeons). One hundred surveys were also distributed to members of the Association of Veterinary Anaesthetists (AVA) to address the question of whether additional training in anaesthesia/analgesia impacted prescribing practice. The survey was also made available online. Reported advantages of producing a written format questionnaire include "*the ability to sample from a larger number of respondents at lower cost in a given time frame compared to, for example, telephone or face-to-face interviews, and a reduced risk of variability in response related to questioning by different interviewers*" (Lavrakas, 2008). Disadvantages include the fact that "*data processing recorded by mail*

questionnaires is laborious and time consuming and there is no ability to control presentation order of items; respondents can examine the contents of the entire instrument before answering any question" (Lavrakas, 2008).

Data from 661 paper questionnaires (completion rate 16.1%) and 59 online questionnaires were analysed, with respondents grouped according to time since graduation, gender, and AVA membership.

The results of the survey enabled us to conclude that significant improvements in analgesic provision had occurred since the previous United Kingdom surveys, but some discrepancies between respondent groups remained. All respondents indicated that at least one opioid and one NSAID analgesic were available, compared with previous data of 91% and 94% respectively (Lascelles et al., 1999). Consistent with previous data (Lascelles et al., 1999; Brodbelt, 2006), buprenorphine remained the opioid most widely available (98.9% of respondents), however 81.8% of respondents stated that they were able to prescribe a full μ opioid agonist in their practice. Around three-quarters of respondents prescribed a combination of opioids and NSAIDs (multi-modal analgesia) to dogs and cats undergoing surgery; significantly more than the 30 per cent of respondents who employed multi-modal analgesia in the previous survey (Capner et al., 1999). Fewer respondents administered perioperative opioids to cats (81.8%) undergoing routine surgeries compared with dogs (90.5%), and fewer respondents provided post discharge NSAIDs to cats (33.4%) compared with dogs (75.1%) following routine surgery, suggesting continued discrepancies in analgesic use between species. Ovariohysterectomy and castration surgeries were considered by respondents to be more painful in dogs compared with cats, which could well account for

the reported differences in prescribing. Male veterinary surgeons were less likely to prescribe perioperative NSAIDs in cats (94.4%) and post-operative NSAIDs in dogs (68.4%) compared to female veterinary surgeons (99.4 % and 78.4%, respectively). Prescribers of perioperative opioids to dogs, and perioperative opioids and NSAIDs to cats, were more recently graduated compared to non-prescribers.

Evaluating the impact of further education or interest in anaesthesia and analgesia, significant differences were identified between AVA members and other respondents. A higher proportion of AVA members (92.6%) prescribed postoperative NSAIDs to dogs (non-AVA 74.3%) and cats (AVA 77.8%, non-AVA 31.5%) undergoing routine surgeries. AVA members were significantly more likely to state that they routinely employed regional local anaesthetic techniques, although they were no more likely than non-members to employ skin infiltration with local anaesthetic. Members of the AVA were also significantly more likely to state that they had previously utilised continuous infusions of analgesics including ketamine, α_2 agonists, lidocaine, and combinations of these with opioids. However, availability of equipment for the controlled delivery of such infusions was reduced for non-AVA members (36.2%) compared with AVA members (100%), which may have reduced the opportunity for non-AVA members to safely utilise such strategies. Interestingly, AVA members were significantly less likely (24.1%) to prescribe tramadol perioperatively to dogs undergoing orthopaedic surgery, compared with non-AVA members (59.5%). Although there are some reports that parenteral tramadol 2mg kg^{-1} provides effective analgesia for orthopaedic surgery in dogs (Yazbek & Fantoni, 2005; Vettorato et al., 2010), oral administration of 4-5 mg kg^{-1} is less effective than NSAIDs alone (Davila et al., 2013). These

findings would be consistent with the pharmacokinetics of tramadol in dogs, which exhibits bioavailability of approximately 30% following oral administration (Giorgi et al., 2009). Higher oral doses (9.9mg kg^{-1}) may be antinociceptive (KuKanich & Papich, 2011). At the time the survey was conducted tramadol prescription would have been as human formulations used 'off-licence' under the cascade provisions of the Veterinary Medicines Regulations. Since completion of the survey tramadol has been added to Schedule 3 of the controlled drugs regulations, and injectable and oral formulations have received marketing authorisation as analgesics for mild post-operative pain and acute and chronic mild soft tissue and musculoskeletal pain in dogs. It is currently unknown what effect, if any, these changes may have had on prescribing.

Pain assessment tools were routinely used by higher proportions of AVA members (dogs 89.3%, cats 64.3%) compared with non-members (14.9% and 15.1%, respectively). Gender and interval since graduation were not associated with the use of pain assessment tools. A higher proportion of pain assessment tool users compared with non-users prescribed perioperative opioids to dogs (96.0% compared with 89.0%) and cats (90.0% compared with 80.0%) undergoing routine surgery, and postoperative NSAIDs to cats (47.5% compared with 30.2%).

This work documented significant improvements in the provision of analgesia to companion animals treated by UK veterinary surgeons over the course of 15 years. The increasing availability of videos demonstrating local anaesthetic techniques, such as those supported by the World Small Animal Veterinary Association[3], would be hoped to improve the uptake

[3] https://www.wsava.org/Committees/Global-Pain-Council accessed 7th March 2019

of these strategies by the veterinary profession, and further surveys should be planned to evaluate the impact of making such continued education available in this form.

Paper

Prescription of perioperative analgesics by UK small animal veterinary surgeons in 2013

J. R. Hunt, T. G. Knowles, B. D. X. Lascelles, J. C. Murrell

Data from a survey conducted in 1996–1997 suggested a low level of perioperative analgesic administration to cats and dogs in the UK. In order to evaluate current practice and attitudes with regards to perioperative analgesic prescription, a cross-sectional survey of UK practising small animal veterinary surgeons was undertaken in spring 2013. Four thousand one hundred paper questionnaires were distributed and the survey was made available online. Seven hundred and twenty valid responses were received and analysed. All respondents had access to at least one non-steroidal anti-inflammatory drug (NSAID) and one opioid within their practice. Respondents considered analgesic efficacy, and degree of intraoperative pain, the most important factors governing their selection of NSAID and opioid analgesics. Perioperative NSAIDs were administered by approximately 98 per cent of respondents to dogs and cats undergoing neutering. Multimodal (opioid+NSAID) analgesia was prescribed by the majority of respondents. Neutering was considered more painful in dogs than in cats, and lower rates of opioid and postdischarge NSAID prescription were reported for cats. Orthopaedic, abdominal and dental surgeries were considered equally painful in dogs and cats. Local analgesic techniques were not commonly used. Analgesic prescription has increased since previous surveys, which should translate to improved animal welfare.

Introduction

In 1999, the results of a survey, conducted between 1996 and 1997, of the attitudes of UK veterinary surgeons to perioperative analgesia in dogs (Capner and others 1999) and cats and small mammals (Lascelles and others 1999) were published. That survey described reduced rates of analgesic use in cats compared with dogs and very low levels of employment of multimodal analgesic strategies. It identified that female veterinary surgeons, and more recently graduated veterinary surgeons, were more likely to use and prescribe analgesic drugs. At that time, there were limited non-steroidal anti-inflammatory drugs (NSAIDs) (carprofen) available with an indication for preanaesthetic administration (Capner and others 1999), and only three opioid analgesics (buprenorphine, butorphanol and pethidine) licensed for use in dogs and/or cats. Studies in South Africa and Canada have demonstrated increases in the prescription of perioperative analgesics for cats and dogs during the periods 2000–2005 (Joubert 2001, 2006) and 1996–2001 (Dohoo and Dohoo 1996a, Hewson and others 2006a), respectively. More recently, a high level of analgesic prescription was reported in a survey of practice during feline neutering surgery in Australia, New Zealand and the UK (Farnworth and others 2014). Increased availability of licensed analgesics, greater awareness of animal pain, greater knowledge of how to treat pain and increasing availability of continuing professional development (CPD) in pain management were identified as factors in these countries that may have increased the proportion of analgesic prescribers. Similar changes have also occurred in the UK since the previous survey was conducted.

Our aim was to survey a representative sample of veterinary surgeons currently involved in treating dogs and cats in the UK about their views towards, and practices in, perioperative analgesia. We hypothesised that perioperative analgesic use would have increased compared with a previous survey of this type (Capner and others 1999, Lascelles and others 1999).

Materials and methods

A cross-sectional survey of veterinary surgeons, engaged in small animal practice in the UK during April and May 2013, was conducted.

A written questionnaire, divided into eight parts (see online supplementary appendix 1), was used. Data about the respondent were collected anonymously in Part I. Part II listed a range of analgesic drugs, and respondents were asked to identify those that were available for perioperative use within the practice. Part III comprised questions regarding the analgesics that the respondent used in dogs. These questions investigated the prescription of perioperative NSAIDs and opioids for routine surgery, and the prescription of postoperative NSAIDs and opioids, use of local and adjunctive analgesics, and specific questions on the analgesic strategy and duration of analgesic treatment that would be typically used for eight different painful

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surgical and medical conditions. Part IV requested that veterinary surgeons assign scores on a numerical rating scale (NRS) corresponding to the degree of pain that they considered each of the eight procedures described in the previous question would elicit, if analgesic drugs were not administered. Parts V and VI mirrored the questions contained in Parts III and IV, but were applied to cats. Part VII requested respondents to indicate whether pain assessment tools were used perioperatively within the practice, and which practice personnel were responsible for perioperative pain assessment in animals. Part VIII asked respondents to indicate whether they believed their knowledge in the area of perioperative analgesia in small animals to be adequate and to indicate their preferred method of updating their knowledge. Parts of the questionnaire were deliberately designed to replicate questions contained within a previous survey (Capner and others 1999, Lascelles and others 1999), in order for direct comparisons to be made.

Four thousand questionnaires were distributed by post via a commercial mailing company (Vetfile, UK) to veterinary surgeons engaged in small animal practice, randomly selected from the company's database. Freepost envelopes to return the survey to the investigators were enclosed. One hundred of the questionnaires were distributed at the Association of Veterinary Anaesthetists (AVA) Spring Meeting in April 2013. The survey was made available online. Before distribution of the questionnaires, a letter informing veterinary surgeons about the purpose of the study was published in the Veterinary Record and Veterinary Times; a link to the online survey was provided (Murrell and others 2013).

Data from completed questionnaires were entered into a spreadsheet (Microsoft Excel, 2003). Data from questionnaires that were partially completed were entered into the spreadsheet if the respondent had provided details on date of graduation or gender. Data from the online questionnaire were exported into a spreadsheet file (Microsoft Excel, 2003), which was then used to populate the main spreadsheet.

Statistical analysis

Descriptive statistics were used to evaluate the demographic data, which was compared with reported data for the UK veterinary profession, and to document the number of respondents with access to analgesics within their practices. Data unsuitable for parametric statistical tests, such as the importance assigned to different factors that influenced prescribing of analgesics and pain scores assigned by respondents, were analysed using Kruskall-Wallis tests and, posthoc, Dunn's multiple comparison test. Differences in prescribing between proportions of male and female respondents, for dogs compared with cats, and AVA members and non-members were analysed using Fisher's exact test or χ^2 tests as appropriate. A general linear model, and posthoc Bonferroni testing, was used to evaluate the effect of increasing time since graduation on responses. Spearman's correlation was used to evaluate the relationship between the interval since graduation and pain scores assigned by respondents. Interaction between the factors 'time since graduation', 'gender' and 'AVA membership' was evaluated using logistic regression. No interaction was identified between factors; therefore, these main effects are individually reported.

Proportions of responses from different categories of respondents are presented as percentages, interval since graduation is presented as mean±SD (years). Non-normally distributed data (eg pain scores and importance of considerations for prescription of analgesic drugs) are summarised using the median (interquartile range).

Results

Demographic data

Of the 4100 written questionnaires distributed, 665 were returned. Data from four questionnaires were excluded from analysis. Response rate to the written questionnaire was therefore 16.2 per cent, and completion rate was 16.1 per cent. The online questionnaire produced 59 usable responses; therefore, the data presented are derived from 720 respondents. The breakdown of responses by demographics is shown in Table 1.

Mean interval since graduation of female respondents (13.1±9.5 years) was less than that of male respondents (21.8±11.3 years, P≤0.0001). The distribution of interval since graduation of survey respondents (Fig 1) appeared to mirror the distribution of age groups among practising UK veterinary surgeons (www.rcvs.org.uk/publications/rcvs-facts-2013/?destination=%2Fpublications%2F). Four hundred and sixty-four (65.2 per cent of 711 respondents who supplied an answer) indicated that they worked in a practice that participated in the RCVS Practice Standards Scheme; 25 (5.4 per cent) indicated the practice was Tier 1, 279 (60.1 per cent) Tier 2 and 103 (22.2 per cent) Tier 3. Fifty-seven respondents did not indicate at which tier the practice was registered.

The proportions of respondents with access to specific analgesic drugs in their practice are shown in Table 2.

All respondents indicated that at least one opioid and one NSAID analgesic were available. Five hundred and eighty-nine (81.8 per cent) respondents indicated that at least one full μ opioid agonist was available in their practice.

The ranked order of importance of factors in the choice of perioperative NSAID and opioid drugs by respondents is shown in Figs 2 and 3 for dogs and Figs 4 and 5 for cats. Respondents determined importance of factors by assigning a value using an NRS, which ranged from 0 to 3; 0 indicating not at all important and 3 indicating of highest importance. Analgesic efficacy was the most highly ranked consideration regarding NSAID prescription in dogs and cats (3 (3–3) P≤0.0001); analgesic efficacy and degree of intraoperative pain were the most highly ranked considerations regarding opioid prescription in dogs and cats (3 (3–3) P≤0.0001).

Administration of analgesics for routine surgeries

Routine surgeries were defined as those performed for neutering and lump removal. Approximately ninety-eight per cent of all respondents administered perioperative (preoperative or intraoperative) NSAIDs to dogs and cats for routine surgeries. In dogs, the proportion of male and female NSAID prescribers was similar (P=0.31). In cats, a lower proportion (94.4 per cent) of male veterinary surgeons prescribed NSAIDs, compared with female veterinary surgeons (99.4 per cent, P≤0.0001).

Perioperative opioids were prescribed by 90.5 per cent of all respondents to dogs undergoing routine surgery; a lower proportion of respondents prescribed opioids to cats (81.8 per cent; P≤0.0001) Within each species, similar proportions of male and female respondents prescribed opioids.

A higher proportion of respondents prescribed postoperative NSAIDs, for continuing analgesia following discharge from the clinic, to dogs undergoing routine surgery, compared with cats (dogs 75.1 per cent; cats 33.4 per cent, P≤0.0001). In dogs, a higher proportion of female veterinary surgeons (78.4 per cent) prescribed postoperative NSAID analgesia for routine surgeries than male veterinary surgeons (68.4 per cent P=0.004); this difference was not apparent in prescriptions of postoperative NSAIDs to cats.

Time since qualifying as a veterinary surgeon was not a significant factor in prescription of perioperative or postoperative NSAIDs to dogs undergoing routine surgery, but prescribers of perioperative opioids to dogs had a shorter time since graduation

TABLE 1: Number and categorisation of respondents

	Overall	Male	Female
Survey respondents	720 (100%)	239 (33.2%)	478 (66.4%)
AVA members	30 (4.2%)	11 (36.7%)	19 (63.3%)
Length of time since graduation (years)	15.8±10.9	21.8±11.3	13.1±9.5

AVA, Association of Veterinary Anaesthetists

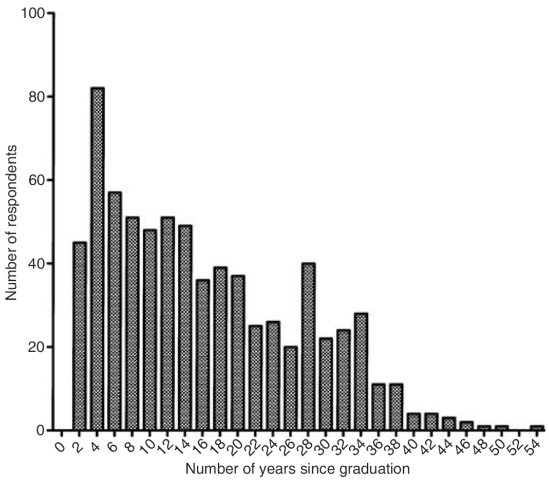

FIG 1: Frequency histogram showing number of respondents (y-axis) by time since graduation (x-axis)

(15.8±12.0 years), compared with non-prescribers (19.7 ±11.4 years; P=0.011). In cats, there was a significant difference in time since graduation between prescribers and non-prescribers of perioperative NSAIDs (15.5±10.6, 31.0±14.3 years; P≤0.0001) and perioperative opioids (15.3±10.5, 18.2±12.3 years; P=0.017).

There were no significant differences between members and non-members of the AVA with regard to perioperative NSAID or opioid administration to dogs and cats for routine surgeries, but a higher proportion of AVA members prescribed postoperative NSAIDs to dogs (AVA 92.6 per cent, non-AVA 74.3 per cent; P=0.03) and cats (AVA 77.8 per cent, non-AVA 31.5 per cent; P≤0.0001).

Local analgesia and constant rate infusion analgesia

The majority of respondents did not use local analgesic techniques routinely (Table 3). Members of the AVA were more likely to use local anaesthetic techniques (P=0.001). In general, prescribers of local analgesic techniques had a shorter time since graduation than non-prescribers.

Adjunctive constant rate infusion analgesia

In dogs, 30 per cent of respondents had administered ketamine constant rate infusions (CRIs) and 23 per cent had administered morphine/lidocaine/ketamine mixture by CRI (Table 3). In cats, 21 per cent of respondents had administered ketamine CRIs for analgesia. A higher proportion of AVA members prescribed adjunctive analgesics compared with non-AVA members (P≤0.0001). Prescribers of CRIs of ketamine, lidocaine and morphine/lidocaine/ketamine in dogs had a significantly shorter interval since graduation than non-prescribers, but this was not different in cats.

Analgesia and pain scores by procedure

Respondents indicated their typical perioperative analgesic strategy for a range of surgeries in dogs (see online supplementary Table S1) and cats (see online supplementary Table S2) and indicated the degree of pain that they considered would be associated with each surgery if no analgesia was provided (see online supplementary Table S3 and Fig 6).

Consistent with the results for the general approach to analgesia for routine surgeries, there was a high level of prescription of perioperative opioids and NSAIDs and postoperative NSAIDs.

Respondents who prescribed opioid and NSAID analgesics concomitantly (multimodal analgesia) for the specified conditions were more recently graduated than those who did not. Members of the AVA were more likely to prescribe multimodal techniques incorporating three or more different analgesic strategies, including the use of local anaesthetic techniques, for all the named procedures. However, an increased proportion of non-AVA members (59.5 per cent) reported the perioperative prescription of tramadol for dogs undergoing orthopaedic surgery, compared with AVA members (24.1 per cent; P≤0.0001). For each procedure, only a very small proportion of respondents indicated that they would not prescribe perioperative analgesics.

Median and interquartile pain scores assigned by respondents are shown in online supplementary Table S3; mean pain scores are illustrated in Fig 6. The pain scores assigned to most procedures varied between individual respondents. Comparison of procedures between dogs and cats identified no interspecies differences in pain scores assigned to orthopaedic or abdominal surgery, dental extractions or pancreatitis. Ovariohysterectomy (dogs 7 (6–8), cats 6 (5–7), P≤0.0001) and castration (dogs 5 (4–6), cats 5 (3–6), P=0.03) were considered more painful in dogs than in cats. Pain scores assigned by prescribers of perioperative opioids and NSAIDs and postoperative NSAIDs were not invariably higher than pain scores assigned by non-prescribers (see online supplementary Table S3). Time since graduation did not correlate with pain scores assigned for any of the procedures (r_s=−0.06 to 0.09).

Pain assessment tools were routinely used by 17 per cent of respondents in dogs and cats. Higher proportions of AVA members used pain assessment tools (dogs 89.3 per cent, cats 64.3 per cent) compared with non-members (14.9 per cent and 15.1 per cent, respectively, P<0.0001 in both cases). There was no association with the proportion of respondents using pain assessment tools and gender, or interval since graduation. A higher proportion of pain assessment tool users compared with non-users prescribed perioperative opioids to dogs (96.0 per cent v 89.0 per cent; P=0.018) and cats (90.0 per cent v 80.0 per cent; P=0.009) undergoing routine surgery and postoperative NSAIDs to cats (47.5 per cent v 30.2 per cent; P≤0.0001).

Comparison of the proportion of analgesic prescribers for comparable surgical procedures between the present survey and

TABLE 2: Proportion of respondents indicating that named analgesics are available within the practice

Opioids	Buprenorphine 98.9%	Butorphanol 96.3%	Methadone (57.3%)	Morphine 49.6%	Fentanyl patch 32.3%	Pethidine 32.0%	Fentanyl injectable 10.3%	Fentanyl transdermal solution 3.2%
NSAIDs	Meloxicam 99.7%	Carprofen 90.4%	Firocoxib 52.1%	Robenacoxib 33.2%	Cimicoxib 22.1%	Ketoprofen 14.2%	Tolfenamic acid 6.1%	
Local anaesthetics	Lidocaine 90.1%	Bupivacaine 36.9%	Mepivicaine 16.1%	Ropivicaine 3.7%				
Adjunctive analgesics	Tramadol 97.6%	Ketamine 97.0%	Dexmedetomidine/ medetomidine 93.7%	Gabapentin 68.6%	Predno-leucotropin 57.9%	Paracetamol/ codeine 51.3%	Aspirin 42.7%	Paracetamol injectable 5.1%

NSAIDs, non-steroidal anti-inflammatory drugs

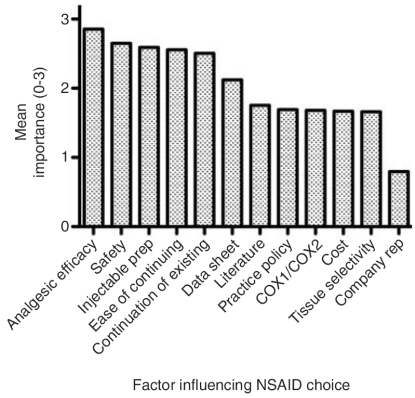

FIG 2: Mean importance attributed by respondents to factors influencing non-steroidal anti-inflammatory drug (NSAID) choice in dogs, 0 indicating not at all important and 3 indicating very important. Factors considered were analgesic efficacy, availability of an injectable preparation, data sheet (Summary of Product Characteristics) indications, COX 1/COX 2 selectivity, tissue selectivity, reported safety (side effects and tolerance), continuation of the same NSAID if patient is on long-term therapy, available product literature/information, cost, practice purchasing policy, relationship with company representative, and ease of continuing therapy after discharge (eg formulation, palatability)

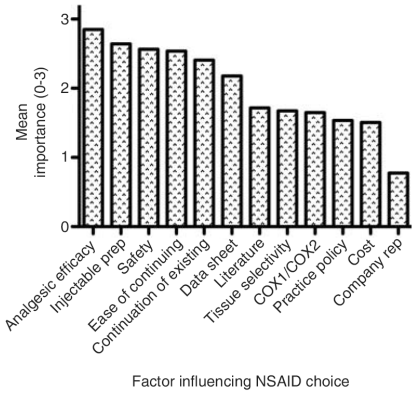

FIG 4: Mean importance attributed by respondents to factors influencing NSAID choice in cats, 0 indicating not at all important and 3 indicating very important. Factors considered were analgesic efficacy, availability of an injectable preparation, data sheet (Summary of Product Characteristics) indications, COX 1/COX 2 selectivity, tissue selectivity, reported safety (side effects and tolerance), continuation of the same NSAID if patient is on long-term therapy, available product literature/information, cost, practice purchasing policy, relationship with company representative, and ease of continuing therapy after discharge (eg formulation, palatability)

the previous UK survey (Capner and others 1999, Lascelles and others 1999) indicated a significantly higher proportion of respondents in the present survey prescribed analgesics ($P \leq 0.0001$).

Both veterinary surgeons and nurses were reported to be responsible for perioperative pain assessment by 85 per cent of respondents; veterinary surgeons alone were reported by 11 per cent and nursing staff alone were reported by four per cent of respondents to be responsible for pain assessment.

Discussion

An increased proportion of respondents employed analgesics perioperatively and postoperatively in both dogs and cats, compared with previous UK results (Capner and others 1999, Lascelles and others 1999). Similar increases in analgesic usage have been identified in sequential surveys of veterinary surgeons in Canada (Hewson and others 2006a) and South Africa (Joubert 2006). Factors which may have contributed to this increased analgesic prescription include increased availability of licensed analgesic drugs for cats and dogs, greater emphasis on analgesia in the undergraduate curriculum and in postgraduate CPD, and client expectations (Demetriou and others 2009).

Results of a survey of pet owners (Demetriou and others 2009) indicated that 77.7 per cent of owners would expect analgesics to be provided routinely for surgery and 61 per cent would expect animals to be sent home with analgesics. The results of the

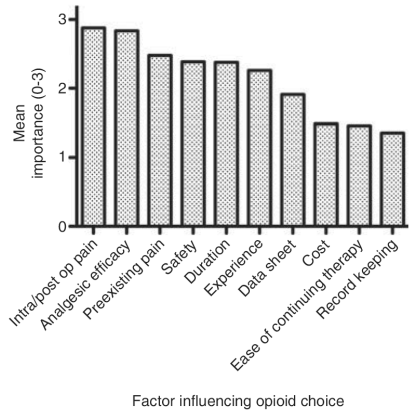

FIG 3: Mean importance attributed by respondents to factors influencing opioid choice in dogs, 0 indicating not at all important and 3 indicating very important. Factors considered were analgesic efficacy, the degree of pain the animal is in before surgery, the degree of pain the animal is likely to be in during or after surgery, data sheet (Summary of Product Characteristics) indications, reported safety (side effects and tolerance), duration of action, cost, record keeping/ storage requirements, experience of using the opioid, and ease of continuing therapy after discharge (eg formulation)

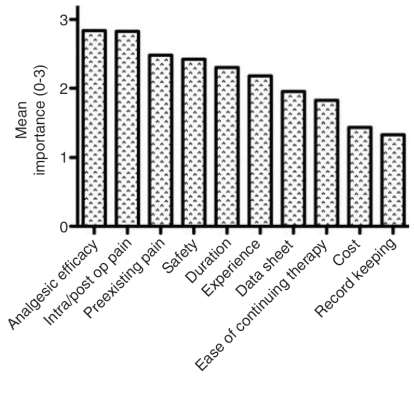

FIG 5: Mean importance attributed by respondents to factors influencing opioid choice in cats, 0 indicating not at all important and 3 indicating very important. Factors considered were analgesic efficacy, the degree of pain the animal is in before surgery, the degree of pain the animal is likely to be in during or after surgery, data sheet (Summary of Product Characteristics) indications, reported safety (side effects and tolerance), duration of action, cost, record keeping/ storage requirements, experience of using the opioid, and ease of continuing therapy after discharge (eg formulation)

TABLE 3: Prescribers of local analgesic techniques (maxillary/mandibular nerve block, periodontal infiltration, skin infiltration, intratesticular infiltration, epidural, brachial plexus block, wound infiltration with local anaesthetic, wound catheter to permit repeated infiltration of local anaesthetic, and radial, ulnar, musculocutaneous, and median (RUMM) nerve block), and adjuvant analgesics by constant rate infusion (CRI) (ketamine, lidocaine, medetomidine, dexmedetomidine, morphine/lidocaine/ketamine and fentanyl/lidocaine/ketamine), to dogs and cats

	Overall proportion of prescribers (dogs) %	Proportion of AVA member prescribers (dogs) %	Proportion of non-AVA member prescribers (dogs) %	P value	Mean (±SD) interval since graduation of prescribers (years)	Mean (±SD) interval since graduation of non-prescribers (years)	P value	Overall proportion of prescribers (cats) %	Proportion of AVA member prescribers (cats) %	Proportion of non-AVA member prescribers (cats) %	P value	Mean (±SD) interval since graduation (years)	Mean (±SD) interval since graduation (years)	P value
Local analgesic techniques														
Maxillary/mandibular	16.6	89.3	13.6	<0.0001*	14.8±10.0	16.4±12.4	0.002*	14.8	86.2	11.7	<0.0001*	14.8±10.2	16.4±12.3	0.653
Periodontal infiltration	12.4	34.4	11.4	0.001*	16.6±10.8	16.1±12.2	0.717	11.5	34.5	10.5	0.001*	16.0±11.0	16.2±12.1	1.000
Skin infiltration	31.7	41.4	31.3	0.31	14.8±10.7	16.8±12.5	0.037*	20.0	37.9	19.1	0.029*	14.7±10.9	16.5±12.3	0.301
Intratesticular	9.0	75.9	6.1	<0.0001*	14.5±11.4	16.3±12.2	0.263	5.5	44.8	3.8	<0.0001*	15.6±10.3	16.2±12.1	1.000
Epidural	13.9	79.3	11.1	<0.0001*	14.8±10.7	16.8±12.5	0.037*	9.2	75.9	6.3	<0.0001*	11.5±7.6	16.6±12.3	0.003*
Brachial plexus	6.7	75.9	3.9	<0.0001*	11.5±7.6	16.6±12.3	0.003*	5.8	58.6	3.9	<0.0001*	11.4±8.0	16.5±12.2	0.025*
Wound infiltration	30.1	69.0	29.1	<0.0001*	16.2±12.5	16.2±11.7	0.990	20.8	51.7	19.6	<0.0001*	16.0±13.4	16.2±11.6	1.000
Wound catheter	6.4	62.1	3.8	<0.0001*	16.4±11.1	16.1±12.1	0.872	4.2	41.4	2.7	<0.0001*	14.3±10.7	16.2±12.1	1.000
RUMM	4.1	55.2	2.1	<0.0001*	12.5±8.6	16.3±12.1	0.090	3.5	48.3	1.8	<0.0001*	11.6±9.2	16.3±12.1	0.144
Constant rate infusions														
Ketamine	30.5	100	27.5	<0.0001*	14.7±9.4	16.8±13.0	0.029*	20.7	89.7	17.7	<0.0001*	15.8±9.8	16.2±12.5	1.000
Lidocaine	18.7	96.6	15.3	<0.0001*	14.0±9.6	16.7±12.5	0.021*	5.1	24.1	4.3	<0.0001*	14.9±10.2	16.2±12.1	1.000
Medetomidine	11.8	79.3	8.9	<0.0001*	16.3±10.2	16.2±12.3	0.940	8.6	55.2	6.6	<0.0001*	16.6±10.4	16.1±12.1	1.000
Dexmedetomidine	3.6	59.3	1.3	<0.0001*	13.5±8.5	16.3±12.1	0.267	2.4	42.9	0.7	<0.0001*	14.1±8.4	16.2±12.1	1.000
Morphine/Lidocaine/Ketamine	23.5	75.9	21.3	<0.0001*	13.6±11.4	16.9±12.1	0.002*	8.9	31.0	8.0	<0.0001*	13.6±9.5	16.4±12.2	0.218
Fentanyl/Lidocaine/Ketamine	3.4	28.6	2.4	<0.0001*	12.1±9.7	16.4±12.1	0.085	1.4	7.1	1.2	0.056	13.7±9.7	16.2±12.1	1.000
Availability of controlled infusion apparatus for delivering CRIs	38.9	100	36.2	<0.0001*	14.4±11.1	17.3±12.1	0.223	28.0	100	25.7	<0.0001*	13.5±9.2	16.3±12.4	0.183

*Significant difference P<0.05
AVA, Association of Veterinary Anaesthetists

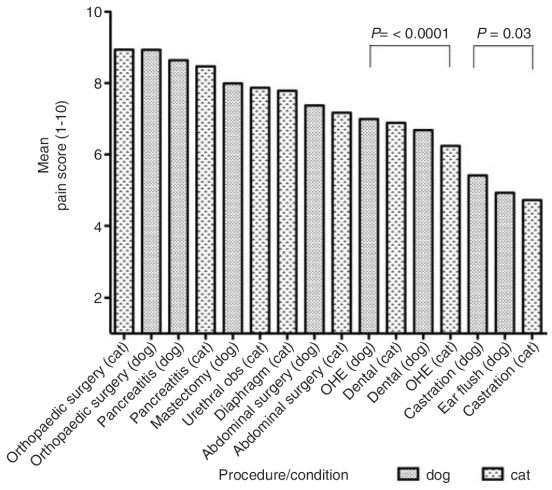

FIG 6: Mean pain scores attributed by respondents to named surgical and medical conditions in dogs and cats using a numerical rating scale which ranged from 1, indicating no pain, to 10 indicating the worst pain imaginable

present survey, if representative, suggest that the veterinary profession is making significant progress in meeting client expectations of analgesic provision perioperatively, and postoperatively to dogs, but has further progress to make in order to meet client expectations in terms of feline postoperative analgesia.

Previous surveys have reported greater prescription of analgesics by female and more recently graduated veterinary surgeons (Dohoo and Dohoo 1996b, Capner and others 1999, Lascelles and others 1999, Hewson and others 2006b), and therefore, the influence of these factors on responses was assessed in the current survey. In the case of prescription of NSAIDs to dogs, or NSAIDs and opioids to cats, undergoing neutering, female and more recently graduated veterinary surgeons remained the groups most likely to prescribe, however, these differences were not consistently identified in other surgeries or medical conditions. Farnworth and others (2014) determined a non-significant effect of the interval since graduation and respondents' gender on the provision of analgesia for feline neutering. Our results indicate that some differences remain in analgesic prescribing practice between male and female veterinary surgeons, and between more recently graduated and more experienced veterinary surgeons, but that these differences appear lessened compared with previous reports, and overall prescription rates are high irrespective of gender and interval since graduation.

Farnworth and others (2014) reported that 98 per cent and 96.2 per cent of respondents prescribed preoperative analgesia to cats undergoing ovariohysterectomy and castration, respectively. The present survey requested data relating to total perioperative use of analgesics, rather than an assessment of preincisional versus postincisional data, but our results were very similar to the findings of Farnworth and others (2014).

We investigated the influence of membership of the AVA on survey responses. Veterinary surgeon members of the AVA have an interest in anaesthesia and analgesia, and many hold postgraduate qualifications and are recognised specialists. In most instances, non-AVA members were as likely as AVA members to prescribe opioid and NSAID analgesics, though prescription of local and adjunctive analgesia was higher among AVA members, which is likely to reflect further education and training in analgesia. However, the data are likely to suffer from bias related to the caseload of AVA members, who may be more likely to work within referral practices and manage analgesia in a different population of animals, compared with non-AVA members.

Pain scores attributed by respondents to the different conditions were subject to marked individual variation. Neutering procedures were ranked as more painful in dogs than in cats, and the reduced prescription of perioperative opioids and postoperative NSAIDs to cats is likely to be a reflection of this, which suggests continuation of bias that was documented in previous surveys (Capner and others 1999, Lascelles and others 1999), and has also been reported in surveys conducted in other countries (Williams and others 2005, Joubert 2006). Non-prescribers of perioperative NSAIDs for neutering procedures in both dogs and cats also assigned lower pain scores to neutering compared with prescribers.

A minority of respondents used pain-assessment tools. An increased proportion of respondents who used pain-scoring tools prescribed perioperative opioids to dogs and cats undergoing routine surgery. Given that use of pain-scoring tools was not influenced by sex or interval since graduation, it is likely that use of pain scales represents an additional factor which influences the decision to prescribe perioperative opioids, which may reflect an increased likelihood of detecting pain in these cases. Pain assessment was reported to be the responsibility of both veterinary surgeons and nurses by the majority of respondents; education and training in pain assessment is therefore essential for both professions.

Approximately three-quarters of respondents prescribed a combination of opioids and NSAIDs (multimodal analgesia) to dogs and cats undergoing surgical procedures, a figure that is significantly higher than the 30 per cent of respondents did this according to the previous survey (Capner and others 1999). Multimodal analgesia is well recognised to improve pain management in human beings (Buvanendran and Kroin 2009, White and others 2009) and cats (Steagall and others 2009), although data are lacking in dogs (Dzikiti and others 2006, Shih and others 2008). The use of local analgesic techniques (Hendrix and others 1996, Huuskonen and others 2013) and adjuncts (Sarrau and others 2007) has the potential to contribute to multimodal analgesia.

Despite the lack of a veterinary licensed oral or injectable formulation of tramadol in the UK and very limited clinical data to support the efficacy of oral tramadol for management of acute pain in cats and dogs (eg Davila and others 2013), it was prescribed by more than 50 per cent of respondents to dogs undergoing orthopaedic surgery and by more than 20 per cent of respondents to dogs undergoing abdominal surgery and mastectomy.

The duration of postoperative pain in dogs and cats undergoing surgery has not been extensively studied. Owners of dogs (Wagner and others 2008) and cats (Väisänen and others 2007) reported behavioural changes, which may be indicative of pain, for up to three days postneutering surgery. A decrease in mechanical nociceptive threshold at the wound, indicating peripheral sensitisation, was reported for up to 72 hours following ovariohysterectomy in dogs (Hancock and others 2005). In the current survey, approximately two-thirds of respondents prescribed postoperative NSAIDs for longer than 24 hours postsurgery for canine neutering procedures. This figure was lower in cats, with only a quarter of respondents prescribing NSAIDs for longer than 24 hours after castration. The recent survey by Farnworth and others (2014) indicated a much lower prevalence of prescription of postdischarge analgesics for feline neutering than was indicated by the responses to the present survey. However, a number of respondents to the current survey indicated that they considered a single subcutaneous dose of carprofen to provide up to 72 hours analgesia in cats, and these respondents may have also indicated that they provided NSAID analgesia for greater than 24 hours postoperatively, potentially artificially increasing the number of prescribers of postoperative NSAIDs in cats. Although the pharmacokinetics of carprofen in cats include a prolonged elimination half-life (Taylor and others 1996, Parton and others 2000), no published studies have evaluated the analgesic effect of carprofen beyond 24 hours following a single dose in cats.

The response and completion rates (16 per cent) to the written questionnaire were lower than the previously conducted survey which reported a 48 per cent return rate (Capner and others 1999, Lascelles and others 1999). The inclusion of online responses to the current survey meant that an overall response rate could not be calculated, as we were unable to determine the number of veterinary surgeons who accessed the online survey. However, despite the lower response rate, the number of surveys distributed was higher than in the 1996 survey; therefore, the number of responses (720) to the current survey represents 75 per cent of the total number of responses (958) to the previous one.

The number of practising veterinary surgeons in the UK in 2013 was 18,413 (www.rcvs.org.uk/publications/rcvs-facts-2013/?destination=%2Fpublications%2F); therefore, respondents to the current survey represent 3.9 per cent of the UK total. Sending out reminder notices might have increased the response rate but was considered cost prohibitive. Responses were accepted for six weeks, shorter than the five-month period of data collection for the 1996 survey, which might have also contributed to the lower return rate. Additionally, the current survey was a 15-sided document, compared with the eight-sided survey described by Capner and others (1999); it is possible that the length of the current survey discouraged some recipients from completing it.

Despite the lower return rate, the range and distribution of interval since graduation of the survey respondents appeared appropriate, considering the age group demographics of the UK veterinary profession. Compared with the overall UK figures, male veterinary surgeons were under-represented in our survey (33 per cent of responses, compared with 44 per cent of the profession). As a consequence, where a sex-related difference in analgesic prescription exists, the overall proportion of prescribers calculated from the survey responses may overestimate the proportion of prescribers within the profession.

Veterinary surgeons with a special interest in analgesia may have been more likely to complete the survey. Although some responses were received from veterinary surgeons who did not routinely employ analgesics, a bias may exist towards respondents more likely to prescribe analgesia, and therefore, results should be interpreted as a 'best-case' scenario. Despite these caveats, the results of the survey are hopefully broadly representative of current views and practice within the UK veterinary profession. The magnitude of changes observed, compared with the previous survey, is extremely unlikely to be entirely attributable to a skewed sample population, and therefore, we believe that these results reflect real increases in the provision of perioperative analgesia to animals.

In conclusion, this study suggests a marked increase in the prescription of perioperative analgesics among veterinary surgeons since 1996. Gender and time since graduation appear to be less associated with analgesic use than previously. Further work to determine the effectiveness of current analgesic strategies for the days following surgery is essential to optimise the welfare of dogs and cats undergoing surgery.

Acknowledgements

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		Orthopaedic surgery	Abdominal surgery	Ovario-hysterectomy	Castration	Ear flush for otitis externa	Dental with extractions	Acute pancreatitis	Mastectomy
Perioperative opioid	Number of prescribers / number of respondents	635/649 (97.8 %)	647/687 (94.2 %)	611/686 (89.1 %)	589/687 (85.7 %)	550/688 (80.0 %)	607/686 (88.5 %)	627/684 (91.7 %)	638/683 (93.4 %)
	Male	214/222 (96.3 %)	207/226 (91.6 %)	198/225 (88.0 %)	196/225 (87.1 %)	179/226 (79.2 %)	197/226 (87.2 %)	201/225 (89.3 %)	209/224 (93.3 %)
	Female	418/424 (98.6 %)	438/458 (95.6 %)	411/458 (89.7 %)	391/458 (85.4 %)	369/459 (80.4 %)	408/457 (89.3 %)	423/456 (92.8 %)	426/456 (93.4 %)
	P	0.088	0.036*	0.514	0.727	0.761	0.444	0.142	1.000
	AVA	29/29 (100.0 %)	29/29 (100 %)	29/29 (100.0 %)	29/29 (100.0 %)	29/29 (100.0 %)	28/29 (96.6 %)	29/29 (100.0 %)	28/28 (100.0 %)
	Non-AVA	606/619 (97.9 %)	618/657 (94.1 %)	582/656 (88.7 %)	560/657 (85.2 %)	521/658 (79.2 %)	579/656 (88.3 %)	598/654 (91.4 %)	610/654 (93.3 %)
	P	1.000	0.401	0.062	0.024*	0.003*	0.236	0.159	0.248
	Length of experience since graduation (years)	Prescribers 15.8 ± 11.9 Non-prescribers 27.7 ± 10.2	Prescribers 15.7 ± 11.9 Non-prescribers 21.3 ± 12.3	Prescribers 15.6 ± 11.8 Non-prescribers 19.2 ± 12.5	Prescribers 15.6 ± 11.8 Non-prescribers 18.4 ± 12.5	Prescribers 15.7 ± 11.9 Non-prescribers 17.5 ± 11.9	Prescribers 15.5 ± 11.8 Non-prescribers 19.8 ± 12.5	Prescribers 15.6 ± 11.8 Non-prescribers 20.5 ± 12.8	Prescribers 15.7 ± 11.8 Non-prescribers 20.2 ± 13.4
	P	<0.0001*	0.004*	0.008*	0.033*	0.114	0.003*	0.003*	0.016*
Perioperative NSAID	Number of prescribers / number of respondents	643/649 (99.1 %)	651/687 (94.8 %)	675/686 (98.4 %)	676/687 (98.4 %)	510/687 (74.2 %)	671/686 (97.8 %)	288/684 (42.1 %)	670/683 (98.1 %)
	Male	218/222 (98.2 %)	218/226 (96.5 %)	218/225 (96.9 %)	218/226 (96.5 %)	163/226 (72.1 %)	218/226 (96.5 %)	107/225 (47.6 %)	219/224 (97.8 %)
	Female	422/424 (99.5 %)	430/458 (93.9 %)	454/458 (99.1 %)	455/458 (99.3 %)	345/458 (75.3 %)	450/457 (98.5 %)	181/456 (39.7 %)	448/456 (98.2 %)
	P	0.189	0.202	0.047*	0.008*	0.403	0.102	0.058	0.767
	AVA	29/29 (100.0 %)	26/29 (89.7 %)	29/29 (100.0 %)	29/29 (100.0 %)	25/29 (86.2 %)	27/29 (93.1 %)	8/29 (27.6 %)	26/28 (92.9 %)
	Non-AVA	614/619 (99.2 %)	624/657 (95.0 %)	646/656 (98.5 %)	646/657 (98.3 %)	484/657 (73.7 %)	643/656 (98.0 %)	279/654 (42.7 %)	644/654 (98.5 %)
	P	1.000	0.190	1.000	1.000	0.191	0.129	0.126	0.083
	Length of experience since graduation (years)	Prescribers 15.9 ± 11.9 Non-prescribers 26.9 ± 18.7	Prescribers 16.1 ± 12.0 Non-prescribers 14.2 ± 11.5	Prescribers 15.9 ± 11.8 Non-prescribers 26.9 ± 15.9	Prescribers 15.8 ± 11.8 Non-prescribers 26.2 ± 15.9	Prescribers 16.3 ± 12.2 Non-prescribers 15.2 ± 11.1	Prescribers 15.9 ± 11.8 Non-prescribers 21.5 ± 15.9	Prescribers 18.0 ± 13.1 Non-prescribers 14.5 ± 10.7	Prescribers 15.9 ± 11.8 Non-prescribers 20.6 ± 18.4
	P	0.026*	0.338	0.002*	0.004*	0.262	0.071	<0.0001*	0.164
Perioperative local analgesia	Number of prescribers / number of respondents	174/649 (26.8 %)	55/686 (8.0 %)	40/686 (5.8 %)	47/687 (6.8 %)	18/686 (2.6 %)	122/686 (17.9 %)	15/683 (2.2 %)	79/682 (11.6 %)
	Male	63/222 (28.4 %)	17/226 (7.5 %)	15/225 (6.7 %)	17/226 (7.5 %)	9/226 (4.0 %)	46/226 (20.4 %)	6/225 (2.7 %)	24/224 (10.7 %)
	Female	109/424 (25.7 %)	38/457 (8.3 %)	25/458 (5.5 %)	30/458 (6.6 %)	9/457 (2.0 %)	76/457 (16.6 %)	9/455 (2.0 %)	55/455 (12.1 %)
	P	0.512	0.767	0.603	0.633	0.133	0.244	0.585	0.703
	AVA	25/29 (86.2 %)	20/29 (69.0 %)	11/29 (37.9 %)	20/29 (69.0 %)	6/28 (21.4 %)	28/29 (96.6 %)	12/28 (42.9 %)	20/27 (74.1 %)
	Non-AVA	149/619 (24.1 %)	35/656 (5.3 %)	29/656 (4.4 %)	27/657 (4.1 %)	12/657 (1.8 %)	94/656 (14.3 %)	3/654 (0.5 %)	59/654 (9.0 %)
	P	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	Length of experience since graduation (years)	Prescribers 13.9 ± 9.2 Non-prescribers 16.8 ± 12.8	Prescribers 13.5 ± 9.6 Non-prescribers 16.2 ± 12.1	Prescribers 14.9 ± 10.9 Non-prescribers 16.1 ± 12.0	Prescribers 13.9 ± 10.7 Non-prescribers 16.2 ± 12.0	Prescribers 14.5 ± 12.2 Non-prescribers 16.1 ± 12.0	Prescribers 14.8 ± 9.5 Non-prescribers 16.3 ± 12.4	Prescribers 14.0 ± 8.4 Non-prescribers 16.1 ± 12.0	Prescribers 15.5 ± 9.9 Non-prescribers 16.1 ± 12.2
	P	0.007*	0.099	0.532	0.201	0.578	0.214	0.505	0.659
Perioperative adjunctive analgesia	Number of prescribers / number of respondents	113/649 (17.4 %)	69/685 (10.1 %)	44/685 (6.4 %)	24/686 (3.5 %)	92/686 (13.4 %)	34/684 (5.0 %)	94/683 (13.8 %)	71/681 (10.4 %)
	Male	42/222 (18.9 %)	25/226 (11.1 %)	15/225 (6.6 %)	12/226 (5.3 %)	26/226 (11.5 %)	14/226 (6.2 %)	40/225 (17.8 %)	27/224 (12.1 %)
	Female	71/424 (16.7 %)	44/456 (9.6 %)	29/457 (6.3 %)	12/457 (2.6 %)	64/457 (14.0 %)	20/455 (4.4 %)	54/455 (11.9 %)	44/454 (9.7 %)
	P	0.514	0.590	0.869	0.080	0.401	0.351	0.044*	0.353
	AVA	21/29 (72.4 %)	18/28 (64.3 %)	9/28 (32.1 %)	8/28 (28.6 %)	16/28 (57.1 %)	10/28 (35.7 %)	20/28 (71.4 %)	14/27 (51.9 %)
	Non-AVA	92/619 (14.9 %)	51/656 (7.7 %)	35/656 (5.3 %)	16/657 (2.4 %)	76/657 (11.6 %)	24/655 (3.7 %)	74/654 (11.3 %)	57/653 (8.7 %)
	P	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

	Length of experience since graduation (years)	Prescribers 16.0 ± 12.3 Non-prescribers 16.1 ± 10.1	Prescribers 15.9 ± 9.3 Non-prescribers 16.0 ± 12.2	Prescribers 15.8 ± 9.4 Non-prescribers 16.1 ± 12.1	Prescribers 17.0 ± 9.2 Non-prescribers 16.0 ± 12.0	Prescribers 15.1 ± 13.2 Non-prescribers 16.2 ± 11.8	Prescribers 17.7 ± 8.8 Non-prescribers 15.9 ± 12.1	Prescribers 15.0 ± 10.3 Non-prescribers 16.2 ± 12.2	Prescribers 15.5 ± 9.5 Non-prescribers 16.1 ± 12.2
	P	0.965	0.950	0.907	0.681	0.417	0.408	0.381	0.683
Perioperative tramadol	Number of prescribers / number of respondents	376/649 (57.9 %)	135/685 (19.7 %)	19/685 (2.8 %)	8/686 (1.2 %)	36/686 (5.2 %)	59/685 (8.6 %)	128/683 (18.7 %)	180/681 (26.4 %)
	Male	124/222 (55.9 %)	47/226 (20.8 %)	6/225 (2.7 %)	2/226 (0.9 %)	11/226 (4.9 %)	23/226 (10.2 %)	35/225 (15.5 %)	58/224 (25.9 %)
	Female	251/424 (59.2 %)	88/456 (19.3 %)	13/457 (2.8 %)	6/457 (1.3 %)	25/457 (5.5 %)	35/456 (7.7 %)	92/455 (20.2 %)	121/454 (26.7 %)
	P	0.450	0.683	1.000	1.000	0.856	0.307	0.174	0.854
	AVA	7/29 (24.1 %)	3/28 (10.7 %)	0/28 (0.0%)	0/28 (0.0 %)	0/28 (0.0 %)	3/28 (10.7 %)	2/28 (7.1 %)	3/27 (11.1 %)
	Non-AVA	368/619 (59.5 %)	132/656 (20.1 %)	19/656 (2.9 %)	8/657 (1.2 %)	36/657 (5.5 %)	56/656 (8.5 %)	126/654 (19.2 %)	177/653 (27.1 %)
	P	<0.0001*	0.331	1.000	1.000	0.391	0.726	0.138	0.075
	Length of experience since graduation (years)	Prescribers 15.3 ± 11.4 Non-prescribers 17.1 ± 12.6	Prescribers 14.2 ± 10.1 Non-prescribers 16.5 ± 12.3	Prescribers 12.8 ± 11.5 Non-prescribers 16.1 ± 12.0	Prescribers 13.7 ± 13.1 Non-prescribers 16.1 ± 11.9	Prescribers 12.3 ± 8.9 Non-prescribers 16.3 ± 12.1	Prescribers 17.4 ± 16.3 Non-prescribers 15.9 ± 11.5	Prescribers 15.0 ± 13.6 Non-prescribers 16.3 ± 11.5	Prescribers 15.6 ± 12.4 Non-prescribers 16.2 ± 11.8
	P	0.051	0.046*	0.232	0.576	0.055	0.367	0.275	0.574
Post-operative NSAID (opioid in the case of pancreatitis) for greater than 24 hours post surgery/ condition	Number of prescribers / number of respondents	621/645 (96.3 %)	557/680 (81.9 %)	525/680 (77.2 %)	434/683 (63.5 %)	348/680 (51.2 %)	540/680 (79.4 %)	409/621 (65.9 %)	608/677 (89.8 %)
	Male	208/221 (94.1 %)	186/225 (82.7 %)	160/223 (71.1 %)	125/224 (55.8 %)	102/223 (45.7 %)	171/224 (76.3 %)	120/209 (57.4 %)	198/222 (89.2 %)
	Female	410/421 (97.4 %)	368/452 (81.4 %)	364/454 (80.2 %)	309/456 (67.8 %)	244/454 (53.7 %)	366/453 (80.8 %)	287/410 (70.0 %)	407/452 (90.0 %)
	P	0.0481*	0.751	0.015*	0.003*	0.060	0.190	0.002*	0.787
	AVA	28/29 (96.6 %)	20/26 (76.9 %)	24/27 (88.9 %)	20/28 (71.4 %)	20/26 (76.9 %)	24/28 (85.7 %)	19/25 (76.0 %)	26/28 (92.9 %)
	Non-AVA	593/615 (96.4 %)	537/653 (82.2 %)	501/652 (76.9 %)	414/654 (63.3 %)	328/653 (50.2 %)	516/651 (79.3 %)	390/595 (65.5 %)	582/648 (89.8 %)
	P	1.000	0.443	0.166	0.429	0.009*	0.484	0.389	1.000
	Length of experience since graduation (years)	Prescribers 15.4 ± 11.0 Non-prescribers 26.3 ± 13.7	Prescribers 15.9 ± 11.9 Non-prescribers 16.5 ± 11.8	Prescribers 15.5 ± 11.9 Non-prescribers 17.7 ± 11.9	Prescribers 15.5 ± 12.3 Non-prescribers 16.8 ± 11.2	Prescribers 15.6 ± 12.4 Non-prescribers 16.5 ± 11.3	Prescribers 15.8 ± 11.9 Non-prescribers 16.4 ± 12.0	Prescribers 14.6 ± 10.1 Non-prescribers 18.2 ± 13.3	Prescribers 15.8 ± 11.8 Non-prescribers 17.5 ± 12.8
	P	<0.0001*	0.592	0.048*	0.161	0.331	0.596	<0.0001*	0.237
NSAID + opioid (multimodal)	Number of prescribers / number of respondents	632/649 (97.4 %)	615/687 (89.5 %)	606/686 (88.3 %)	584/687 (85.0 %)	412/686 (60.1 %)	598/686 (87.2 %)	244/684 (35.7 %)	630/683 (92.2 %)
	Male	213/222 (95.9 %)	202/226 (89.4 %)	195/225 (86.7 %)	192/226 (85.0 %)	132/226 (58.4 %)	193/226 (85.4 %)	90/225 (40.0 %)	207/225 (92.0 %)
	Female	416/425 (97.9 %)	411/458 (89.7 %)	409/458 (89.3 %)	390/458 (85.2 %)	279/459 (60.8 %)	403/457 (88.2 %)	154/456 (33.8 %)	420/456 (92.1 %)
	P	0.207	0.894	0.311	1.000	0.562	0.330	0.126	1.000
	AVA	29/29 (100.0 %)	26/29 (89.7 %)	29/29 (100.0 %)	29/29 (100.0 %)	25/29 (86.2 %)	27/29 (93.1 %)	8/29 (27.6 %)	26/29 (89.7 %)
	Non-AVA	603/620 (97.3 %)	589/657 (89.6 %)	577/656 (88.0 %)	555/657 (84.5 %)	387/658 (58.8 %)	571/656 (87.0 %)	236/654 (36.1 %)	604/654 (92.4 %)
	P	1.000	1.000	0.039*	0.014*	0.003*	0.566	0.431	0.486
	Length of experience since graduation (years)	Prescribers 15.7 ± 11.8 Non-prescribers 26.8 ± 12.7	Prescribers 15.8 ± 11.8 Non-prescribers 18.1 ± 12.7	Prescribers 15.5 ± 11.7 Non-prescribers 20.1 ± 12.9	Prescribers 15.5 ± 11.7 Non-prescribers 19.1 ± 13.1	Prescribers 16.0 ± 12.2 Non-prescribers 16.1 ± 11.6	Prescribers 15.4 ± 11.7 Non-prescribers 20.3 ± 13.1	Prescribers 17.7 ± 13.4 Non-prescribers 15.0 ± 10.9	Prescribers 15.6 ± 11.6 Non-prescribers 20.4 ± 14.4
	P	<0.0001*	0.122	0.001*	0.004*	0.953	<0.0001*	0.005*	0.005*
No perioperative analgesics	Number of respondents who did not prescribe any perioperative analgesic / number of respondents	2/649 (0.3 %)	4/687 (0.6 %)	6/686 (0.9 %)	5/687 (0.7 %)	29/688 (4.2 %)	5/686 (0.7 %)	8/684 (1.2 %)	5/683 (0.7 %)

Supplementary table 1 – Prescribers of classes of analgesic drugs for named surgical or medical conditions in dogs. * = significant difference *P* < 0.05

		Orthopaedic surgery	Ruptured diaphragm repair	Abdominal surgery	Ovario-hysterectomy	Castration	Dental with extractions	Acute pancreatitis	Relief of urethral obstruction
Perioperative opioid	Number of prescribers / number of respondents	616/639 (96.4 %)	600/647 (92.7 %)	602/665 (90.5 %)	548/670 (81.8 %)	525/670 (78.4 %)	578/669 (86.4 %)	590/660 (89.4 %)	580/661 (87.7 %)
	Male	200/215 (93.0 %)	199/218 (91.3 %)	190/220 (86.4 %)	181/220 (82.3 %)	172/220 (78.2 %)	186/220 (84.5 %)	188/216 (87.0 %)	187/217 (86.1 %)
	Female	414/422 (98.1 %)	399/427 (93.4 %)	411/443 (92.8 %)	366/448 (81.7 %)	352/448 (78.6 %)	391/447 (87.5 %)	400/442 (90.5 %)	391/442 (88.5 %)
	P	0.003*	0.338	0.010*	0.915	0.920	0.335	0.180	0.449
	AVA	28/28 (100.0 %)	27/27 (100.0 %)	27/27 (100.0 %)	26/27 (96.3 %)	27/27 (100.0 %)	27/27 (100.0 %)	25/26 (96.2 %)	27/27 (100.0 %)
	Non-AVA	587/610 (96.2 %)	572/619 (92.4 %)	574/637 (90.1 %)	521/642 (81.2 %)	497/642 (77.4 %)	550/641 (85.8 %)	564/633 (89.1 %)	552/633 (87.2 %)
	P	0.617	0.250	0.099	0.043*	0.002*	0.039*	0.509	0.064
	Length of experience since graduation (years)	Prescribers 15.6 ± 11.3 Non-prescribers 24.1 ± 12.3	Prescribers 15.9 ± 11.9 Non-prescribers 18.1 ± 11.9	Prescribers 15.7 ± 12.0 Non-prescribers 19.6 ± 11.4	Prescribers 15.7 ± 12.0 Non-prescribers 17.6 ± 12.0	Prescribers 15.7 ± 12.0 Non-prescribers 17.2 ± 11.8	Prescribers 15.7 ± 12.0 Non-prescribers 18.0 ± 12.1	Prescribers 15.5 ± 11.9 Non-prescribers 19.5 ± 12.2	Prescribers 15.3 ± 11.8 Non-prescribers 20.4 ± 12.1
	P	0.003	0.660	0.027*	0.331	0.548	0.233	0.025*	0.001*
Perioperative NSAID	Number of prescribers / number of respondents	626/639 (98.0 %)	622/647 (96.1 %)	620/665 (93.2 %)	646/670 (96.4 %)	627/670 (93.6 %)	647/669 (96.7 %)	307/660 (46.5 %)	518/661 (78.4 %)
	Male	209/215 (97.2 %)	211/218 (96.8 %)	205/220 (93.2 %)	204/220 (92.7 %)	198/220 (90.0%)	210/220 (95.5 %)	112/216 (51.9 %)	172/218 (78.9 %)
	Female	415/422 (98.3 %)	409/427 (95.8 %)	413/443 (93.2 %)	440/448 (98.2 %)	427/448 (95.3 %)	435/447 (97.3 %)	195/442 (44.1 %)	344/441 (78.0 %)
	P	0.379	0.688	1.000	0.001*	0.011*	0.249	0.067	0.841
	AVA	28/28 (100.0 %)	25/27 (92.6 %)	24/27 (88.9 %)	26/27 (96.3 %)	27/27 (100.0 %)	27/27 (100.0 %)	10/26 (38.5 %)	15/27 (55.5 %)
	Non-AVA	597/610 (97.9 %)	597/619 (96.4 %)	596/637 (93.6 %)	620/642 (96.6 %)	600/642 (93.5 %)	620/641 (96.7 %)	297/633 (46.9 %)	503/633 (79.5 %)
	P	1.000	0.265	0.414	1.000	0.404	1.000	0.429	0.007*
	Length of experience since graduation (years)	Prescribers 15.9 ± 11.4 Non-prescribers 17.8 ± 16.2	Prescribers 16.0 ± 11.7 Non-prescribers 16.3 ± 15.9	Prescribers 16.2 ± 11.8 Non-prescribers 14.8 ± 13.9	Prescribers 15.6 ± 11.7 Non-prescribers 25.7 ± 15.3	Prescribers 15.6 ± 11.7 Non-prescribers 22.0 ± 14.7	Prescribers 15.8 ± 11.8 Non-prescribers 20.7 ± 16.4	Prescribers 17.5 ±13.1 Non-prescribers 14.5 ± 10.7	Prescribers 16.1 ± 12.1 Non-prescribers 15.8 ± 11.8
	P	0.174	1.000	0.421	<0.0001*	0.002*	0.182	0.002*	1.000
Perioperative local analgesia	Number of prescribers / number of respondents	119/637 (18.7 %)	35/647 (5.4 %)	38/665 (5.7 %)	15/670 (2.2 %)	22/670 (3.3 %)	93/669 (13.9 %)	13/655 (2.0 %)	42/661 (6.3 %)
	Male	43/215 (20.0 %)	10/218 (4.6 %)	16/220 (7.3 %)	8/220 (3.6 %)	9/220 (4.1 %)	36/220 (16.4 %)	5/215 (2.3 %)	17/217 (7.8 %)
	Female	75/420 (17.9 %)	25/427 (5.8 %)	22/443 (5.0 %)	7/448 (1.6 %)	12/448 (2.7 %)	57/447 (12.8 %)	8/438 (1.8 %)	25/442 (5.6 %)
	P	0.519	0.584	0.286	0.100	0.490	0.234	0.767	0.310
	AVA	22/27 (81.5 %)	9/27 (33.3 %)	13/27 (48.1 %)	4/27 (14.8 %)	10/27 (37.0 %)	21/27 (77.8 %)	8/26 (30.8 %)	14/27 (51.9 %)
	Non-AVA	97/609 (15.9 %)	26/619 (4.2 %)	25/637 (3.9 %)	11/642 (1.7 %)	12/642 (1.9 %)	72/641 (11.2 %)	5/628 (0.8 %)	28/633 (4.4 %)
	P	<0.0001*	<0.0001*	<0.0001*	0.002*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	Length of experience since graduation (years)	Prescribers 13.4 ± 9.3 Non-prescribers 18.1 ± 15.4	Prescribers 16.6 ± 10.6 Non-prescribers 18.1 ± 12.5	Prescribers 15.9 ± 10.8 Non-prescribers 16.1 ± 12.1	Prescribers 20.3 ± 11.6 Non-prescribers 15.9 ± 12.0	Prescribers 16.3 ± 8.9 Non-prescribers 16.0 ± 12.1	Prescribers 14.9 ± 10.0 Non-prescribers 16.2 ± 12.3	Prescribers 16.6 ± 9.4 Non-prescribers 15.9 ± 12.0	Prescribers 17.2 ± 10.0 Non-prescribers 15.9 ± 12.1
	P	0.023*	0.353	0.552	0.491	1.000	0.998	1.000	1.000
Perioperative adjunctive analgesia	Number of prescribers / number of respondents	104/637 (16.3 %)	65/647 (10.0 %)	68/665 (10.2 %)	63/670 (9.4 %)	70/670 (10.4 %)	46/669 (6.9 %)	64/655 (9.8 %)	81/661 (12.3 %)
	Male	35/215 (16.3 %)	25/218 (11.5 %)	24/220 (10.9 %)	21/220 (9.5 %)	22/220 (10.0 %)	18/219 (8.2 %)	24/215 (11.2 %)	22/216 (10.2 %)
	Female	69/420 (16.4 %)	40/427 (9.4 %)	44/443 (9.9 %)	42/448 (9.4 %)	48/448 (10.7 %)	28/447 (6.3 %)	40/438 (9.1 %)	59/442 (13.3 %)
	P	1.000	0.409	0.686	1.000	0.893	0.416	0.405	0.259
	AVA	21/27 (77.8 %)	16/27 (59.3 %)	15/27 (55.6 %)	8/27 (29.6 %)	8/27 (29.6 %)	7/27 (25.9 %)	14/26 (53.8)	8/27 (29.6 %)
	Non-AVA	83/609 (13.6 %)	49/619 (7.9 %)	53/637 (8.3 %)	55/642 (8.6 %)	62/642 (9.7 %)	39/640 (6.1 %)	50/628 (8.0 %)	73/632 (11.6 %)
	P	<0.0001*	<0.0001*	<0.0001*	0.002*	0.004*	0.001*	<0.0001*	0.012*

	Length of experience since graduation (years)	Prescribers 15.4 ± 10.3 Non-prescribers 18.1 ± 15.4	Prescribers 17.9 ± 9.5 Non-prescribers 15.8 ± 12.1	Prescribers 16.7 ± 9.5 Non-prescribers 16.0 ± 12.2	Prescribers 12.1 ± 8.5 Non-prescribers 16.4 ± 12.2	Prescribers 11.4 ± 8.0 Non-prescribers 16.5 ± 12.3	Prescribers 15.2 ± 9.6 Non-prescribers 16.0 ± 12.2	Prescribers 16.2 ± 9.0 Non-prescribers 15.9 ± 12.3	Prescribers 12.6 ± 8.0 Non-prescribers 16.4 ± 12.4
	P	1.000	0.148	0.508	0.021*	0.002*	1.000	1.000	0.022*
Perioperative tramadol	Number of prescribers / number of respondents	101/637 (15.9 %)	34/647 (5.3 %)	25/665 (3.8 %)	1/670 (0.2 %)	0/670 (0.0 %)	25/669 (3.7 %)	54/655 (8.2 %)	25/660 (3.8 %)
	Male	24/215 (11.2 %)	10/218 (4.6 %)	9/220 (4.1 %)	1/220 (0.5 %)	0/220 (0.0 %)	9/220 (4.1 %)	15/215 (7.0 %)	8/216 (3.7 %)
	Female	77/420 (18.3 %)	24/427 (5.6 %)	16/443 (3.6 %)	0/448 (0.0 %)	0/448 (0.0 %)	16/447 (3.6 %)	38/438 (8.7 %)	17/442 (3.8 %)
	P	0.022*	0.710	0.829	0.329	[a]	0.829	0.543	1.000
	AVA	3/27 (11.1 %)	2/27 (7.4 %)	0/27 (0.0 %)	0/27 (0.0 %)	0/27 (0.0 %)	0/27 (0.0 %)	1/26 (3.8 %)	1/27 (3.7 %)
	Non-AVA	98/609 (16.1 %)	32/619 (5.2 %)	25/637 (3.9 %)	1/642 (0.2 %)	0/642 (0.0 %)	25/641 (3.9 %)	53/628 (8.4 %)	24/632 (3.8 %)
	P	0.787	0.647	0.618	1.000	[a]	0.618	0.715	1.000
	Length of experience since graduation (years)	Prescribers 12.9 ± 9.8 Non-prescribers 18.1 ± 15.4	Prescribers 12.4 ± 8.4 Non-prescribers 16.2 ± 12.1	Prescribers 13.0 ± 8.2 Non-prescribers 16.2 ± 12.1	Prescribers 12.2 ± 0.0 Non-prescribers 16.0 ± 12.0	Prescribers N/A Non-prescribers 16.0 ± 12.0	Prescribers 12.6 ± 9.5 Non-prescribers 16.1 ± 12.1	Prescribers 13.1 ± 9.8 Non-prescribers 16.2 ± 12.1	Prescribers 16.1 ± 10.6 Non-prescribers 16.0 ± 12.0
	P	0.015*	0.073	0.232	0.165	[a]	0.455	0.198	1.000
Post-operative NSAID (opioid in the case of pancreatitis) for greater than 24 hours post surgery/ condition	Number of prescribers / number of respondents	589/631 (93.3 %)	567/639 (88.7 %)	527/653 (80.7 %)	302/664 (45.5 %)	162/664 (24.4 %)	517/662 (78.1 %)	388/608 (63.8 %)	449/652 (68.9 %)
	Male	191/212 (90.1 %)	183/215 (85.1 %)	165/214 (77.1 %)	92/216 (42.6 %)	59/216 (27.3 %)	158/216 (73.1 %)	116/201 (57.7 %)	137/214 (64.0 %)
	Female	396/417 (95.0 %)	382/422 (90.5 %)	360/437 (82.4 %)	210/446 (47.1 %)	103/446 (23.1 %)	357/444 (80.4 %)	271/405 (66.9 %)	311/436 (71.3 %)
	P	0.027*	0.047*	0.114	0.281	0.248	0.045*	0.031*	0.071
	AVA	25/27 (92.6 %)	23/26 (88.5 %)	20/26 (76.9 %)	22/27 (81.5 %)	16/27 (59.3 %)	25/27 (92.6 %)	20/24 (83.3 %)	15/26 (57.7 %)
	Non-AVA	564/603 (93.5 %)	544/612 (88.9 %)	507/626 (81.0 %)	280/636 (44.0 %)	146/636 (23.0 %)	492/634 (77.6 %)	368/583 (63.1 %)	434/625 (69.4 %)
	P	0.693	1.000	0.612	<0.0001*	<0.0001*	0.092	0.050	0.202
	Length of experience since graduation (years)	Prescribers 15.6 ± 11.1 Non-prescribers 19.7 ± 14.2	Prescribers 15.7 ± 11.7 Non-prescribers 18.1 ± 12.7	Prescribers 15.9 ± 11.9 Non-prescribers 16.7 ± 12.1	Prescribers 15.6 ± 12.8 Non-prescribers 16.2 ± 11.2	Prescribers 16.7 ± 12.5 Non-prescribers 15.6 ± 11.7	Prescribers 15.5 ± 11.8 Non-prescribers 17.2 ± 12.3	Prescribers 14.9 ± 11.2 Non-prescribers 17.7 ± 13.2	Prescribers 15.4 ± 12.0 Non-prescribers 16.8 ± 11.7
	P	0.026*	0.094	0.499	0.545	0.350	0.126	0.005*	0.155
NSAID + opioid (multimodal)	Number of prescribers / number of respondents	608/639 (95.1 %)	586/647 (90.6 %)	564/665 (84.8 %)	539/670 (80.4 %)	502/670 (74.9 %)	565/669 (84.5 %)	263/662 (39.7 %)	449/661 (67.9 %)
	Male	197/215 (91.6 %)	194/219 (88.6 %)	179/221 (81.0 %)	174/220 (79.1 %)	162/220 (73.6 %)	181/220 (82.3 %)	97/216 (44.9 %)	146/218 (67.0 %)
	Female	409/422 (96.9 %)	390/434 (89.9 %)	384/445 (86.3 %)	364/450 (81.3 %)	339/448 (75.7 %)	383/447 (85.7 %)	166/444 (37.4 %)	301/442 (68.1 %)
	P	0.006*	0.686	0.088	0.606	0.570	0.256	0.075	0.791
	AVA	28/28 (100.0 %)	25/29 (86.2 %)	24/28 (85.7 %)	26/29 (89.7 %)	27/27 (100.0 %)	27/27 (100.0 %)	9/26 (34.6 %)	15/27 (55.6 %)
	Non-AVA	579/611 (94.8 %)	561/625 (89.8 %)	540/639 (84.5 %)	513/642 (79.9 %)	475/642 (74.0 %)	538/641 (83.9 %)	254/635 (40.0 %)	434/634 (68.5 %)
	P	0.389	0.530	1.000	0.239	<0.0001*	0.014*	0.685	0.205
	Length of experience since graduation (years)	Prescribers 15.5 ± 11.2 Non-prescribers 23.2 ± 13.8	Prescribers 15.7 ± 11.7 Non-prescribers 17.7 ± 13.8	Prescribers 15.7 ± 11.8 Non-prescribers 18.1 ± 12.8	Prescribers 15.4 ± 11.7 Non-prescribers 18.4 ± 12.8	Prescribers 15.3 ± 11.7 Non-prescribers 18.0 ± 12.6	Prescribers 15.5 ± 11.7 Non-prescribers 18.9 ± 12.9	Prescribers 17.4 ± 13.3 Non-prescribers 15.0 ± 10.9	Prescribers 15.2 ± 11.9 Non-prescribers 17.6 ± 12.1
	P	<0.0001*	0.193	0.064	0.009*	0.013*	0.009*	0.012*	0.018*
No perioperative analgesics	Number of respondents who did not prescribe any perioperative analgesic / number of respondents	5/639 (0.8 %)	11/647 (1.7 %)	7/665 (1.1 %)	15/670 (2.2 %)	20/670 (3.0 %)	9/669 (1.3 %)	22/660 (3.3 %)	13/661 (2.0 %)

Supplementary table 2 - Prescribers of classes of analgesic drugs for named surgical or medical conditions in cats. * = significant difference *P* < 0.05. [a] = statistics not calculated as zero respondents were prescribers.

	Orthopaedic surgery (dog)	Abdominal surgery (dog)	Ovario-hysterectomy (dog)	Castration (dog)	Ear flush (dog)	Dental with extractions (dog)	Acute pancreatitis (dog)	Mastectomy (dog)	Orthopaedic surgery (cat)	Diaphragmatic rupture repair (cat)	Abdominal surgery (cat)	Ovario-hysterectomy (cat)	Castration (cat)	Dental with extractions (cat)	Acute pancreatitis (cat)	Relief of Urethral obstruction (cat)
Median (interquartile range) pain score	9 (8-10)	7 (7-8)	7 (6-8)	5 (4-6)	5 (3-6)	7 (6-8)	9 (8-10)	8 (7-9)	9 (8-10)	8 (7-9)	7 (6-8)	6 (5-7)	5 (3-6)	7 (6-8)	9 (8-9)	8 (7-9)
Male	9 (8-10)	7 (6-8)	7 (6-8)	5 (4-6)	4 (3-6)	7 (5-8)	9 (8-9)	8 (7-9)	9 (8-10)	7 (6.5-8)	7 (6-8)	6 (5-7)	5 (3-6)	7 (6-8)	8 (8-9)	8 (7-9)
Female	9 (8-10)	8 (7-8)	7 (6-8)	6 (4.75-6)	5 (4-6)	7 (6-8)	9 (8-10)	8 (7-9)	9 (8-10)	8 (7-9)	7 (7-8)	7 (5-7)	5 (3-6)	7 (6-8)	9 (8-10)	8 (7-9)
P	ns	0.01*	0.01*	ns	ns	Ns	ns	ns	ns	<0.0001*	<0.0001*	0.001*	ns	ns	ns	ns
AVA	9 (8-10)	7.5 (7-8)	7 (6-8)	6 (4.5-7)	6 (5-8)	7 (6-8)	9 (8-10)	8 (8-9)	9 (8-10)	7 (7-10)	7 (6.25-8)	7 (5-7)	6 (4.25-6.75)	7 (6.25-8)	9 (8-10)	8 (7-9)
Non-AVA	9 (8-10)	7 (7-8)	7 (6-8)	5 (4-6)	5 (3-6)	7 (6-8)	9 (8-10)	8 (7-9)	9 (8-10)	8 (7-9)	7 (6-8)	6 (5-7)	5 (3-6)	7 (6-8)	9 (8-9)	8 (7-9)
P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Perioperative opioid prescribers	Prescribers 9 (8-10) Non-prescribers 9 (8-10)	Prescribers 7 (7-8) Non-prescribers 7 (6-9)	Prescribers 7 (6-8) Non-prescribers 7 (6-8)	Prescribers 5 (4-6) Non-prescribers 5 (4-6)	Prescribers 5 (4-7) Non-prescribers 3 (2-4)	Prescribers 7 (6-8) Non-prescribers 6 (5-7)	Prescribers 9 (8-10) Non-prescribers 8 (8-9)	Prescribers 8 (7-9) Non-prescribers 8 (7-8)	Prescribers 9 (8-10) Non-prescribers 9 (8-10)	Prescribers 8 (7-9) Non-prescribers 7.5 (7-9)	Prescribers 7 (6-7) Non-prescribers 6 (5-7)	Prescribers 6 (5-7) Non-prescribers 6 (4-7)	Prescribers 5 (4-7) Non-prescribers 4 (4-7)	Prescribers 7 (6-8) Non-prescribers 7 (6-8)	Prescribers 9 (8-9) Non-prescribers 8 (8-9)	Prescribers 8 (7-9) Non-prescribers 8 (6-9)
P	ns	<0.0001*	ns	ns	<0.0001*	0.002*	0.002*	0.012*	ns	0.016*	<0.0001*	0.008*	Ns	<0.0001*	ns	<0.0001*
Perioperative NSAID prescribers	Prescribers 9 (8-10) Non-prescribers 9 (9-10)	Prescribers 7 (7-8) Non-prescribers 7 (6-8)	Prescribers 7 (6-8) Non-prescribers 6 (5-8)	Prescribers 5 (4-6) Non-prescribers 4 (3-6)	Prescribers 5 (4-7) Non-prescribers (4 (3-6)	Prescribers 7 (6-8) Non-prescribers 6.5 (4-8)	Prescribers 9 (8-10) Non-prescribers 9 (8-10)	Prescribers 8 (7-9) Non-prescribers 8 (7-9)	Prescribers 9 (8-10) Non-prescribers 9 (8-10)	Prescribers 8 (7-9) Non-prescribers 7 (6-8)	Prescribers 7 (6-8) Non-prescribers 7 (6-7)	Prescribers 6 (5-7) Non-prescribers 5 (3-6)	Prescribers 5 (4-7) Non-prescribers 3.5 (2-4)	Prescribers 7 (6-8) Non-prescribers 7 (5-8)	Prescribers 9 (8-10) Non-prescribers 9 (8-9)	Prescribers 8 (7-9) Non-prescribers 8 (7-9)
P	ns	0.003*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.016*	ns	ns	<0.0001*	Ns	<0.0001*	0.007*	0.006*	ns	ns
Post-operative NSAID prescribers	Prescribers 9 (8-10) Non-prescribers 9 (8-10)	Prescribers 7 (6-7) Non-prescribers 7 (6-8)	Prescribers 7 (6-8) Non-prescribers 7 (6-8)	Prescribers 6 (5-7) Non-prescribers 5 (4-6)	Prescribers 5 (4-7) Non-prescribers 4 (3-6)	Prescribers 7 (6-8) Non-prescribers 7 (5-8)	Prescribers 9 (8-10) Non-prescribers 9 (8-9)	Prescribers 8 (7-9) Non-prescribers 8 (7-9)	Prescribers 9 (8-10) Non-prescribers 9 (9-10)	Prescribers 8 (7-9) Non-prescribers 7 (6-8)	Prescribers 7 (6-7) Non-prescribers 7 (5-7)	Prescribers 7 (6-7) Non-prescribers 6 (5-7)	Prescribers 5 (4-6) Non-prescribers 5 (3-6)	Prescribers 7 (6-8) Non-prescribers 7 (5-8)	Prescribers 9 (8-9) Non-prescribers 8 (8-9)	Prescribers 8 (7-9) Non-prescribers 8 (7-9)
P	ns	ns	0.025*	ns	<0.0001*	<0.0001*	ns	ns	ns	0.004*	0.001*	0.002*	0.002*	<0.0001*	ns	ns

Supplementary table 3 – Median (interquartile range) pain scores assigned by categories of respondents for named surgical and medical conditions. * = significant difference *P* < 0.05

University of BRISTOL

Bristol University survey on the administration of analgesics in the peri-operative period to cats & dogs

Peri-operative is defined as the period **from premedication/ induction of anaesthesia until 24 hours after surgery**

Please circle/ tick as appropriate or answer in the grey boxes provided

Part I

1. Your year of graduation from veterinary school:

2. Your sex (please circle): Male Female

3. Practice details:

a. In a typical working week what is your percentage of time spent treating:

i. Dogs

ii. Cats

b. Is the practice that you work in (please circle):

i. Small animal only

ii. Mixed

c. Does your practice participate in the RCVS practice standards scheme (please circle):

i. Yes

ii. No

…… If yes, at which tier is the practice registered:

4. Your level of continuing education (please indicate all that apply & specify discipline):

RCVS Certificate	
Certificate from another CPD provider	
Internship	
Residency	
RCVS or European Diploma	
Ongoing CPD according to or exceeding RCVS requirements	

5. Are you a member of the Association of Veterinary Anaesthetists (AVA) (please circle):

Yes No

6. In which county is your practice located:

Part II

Please indicate (with a tick in the box) whether the following drugs are available in your practice to provide peri-operative analgesia in cats & dogs

Opioids		**NSAIDs**		**Local anaesthetics**		**Adjunctive/non-traditional analgesics**	
Buprenorphine		Carprofen		Bupivicaine		Aspirin	
Butorphanol		Cimicoxib		Lidocaine		Dexmedetomidine or medetomidine	
Fentanyl injectable		Firocoxib		Mepivicaine		Gabapentin	
Fentanyl transdermal patch		Ketoprofen		Ropivacaine		Ketamine	
Fentanyl transdermal spot-on		Meloxicam				Paracetamol for injection	
Methadone		Robenacoxib				Paracetamol/codeine (oral)	
Morphine		Tolfenamic acid				PLT tablets	
Pethidine						Tramadol	
						Other (please specify)	

3

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Part III

The following questions relate to your use of analgesics in the peri-operative period in DOGS:

A: Administration of NSAIDs

1. **Do you typically administer NSAIDs in the peri-operative period to healthy dogs undergoing routine procedures (e.g. lump removal, ovariohysterectomy, castration)?**

   YES NO

2. **If YES, in the peri-operative period, in an individual healthy dog when do you typically administer the NSAID? (please tick one and state route of administration – subcutaneous [SC], intramuscular [IM], intravenous [IV], per os [PO]).**

Before or at the time of premedication		At the end of anaesthesia during the recovery period	
After induction of anaesthesia or during surgery		Other (please specify time & route)	

3. **Do you routinely send dogs that have undergone <u>elective</u> ovariohysterectomy home with ongoing NSAID oral therapy (please circle)?**

   YES NO

   i. **If YES, for how many days do you typically continue oral NSAID therapy? (number of days):**

4. **When considering *which* NSAID to give in the peri-operative period to dogs how important (on a scale of 0 [not important] to 3 [very important]) do you rate the following factors (please circle)?**

	Not Important			Very Important
Analgesic efficacy	0	1	2	3
Availability of an injectable preparation	0	1	2	3
Data sheet (SPC) indications	0	1	2	3
COX 1/ COX 2 selectivity	0	1	2	3
Tissue selectivity	0	1	2	3
Reported safety (side effects & tolerance)	0	1	2	3
Continuation of same NSAID if patient is on long term therapy	0	1	2	3
Available product literature / information	0	1	2	3
Cost	0	1	2	3
Practice purchasing policy	0	1	2	3
Relationship with company representative	0	1	2	3
Ease of continuing therapy after discharge (e.g. formulation, palatability)	0	1	2	3

B: Administration of opioids

1. **Do you typically administer opioids in the peri-operative period to healthy dogs undergoing routine procedures (e.g. lump removal, ovariohysterectomy, castration)?**

   YES NO

2. **If YES, in the peri-operative period, in an individual healthy dog when do you typically administer the opioid? (please tick ALL that apply and state route of administration – subcutaneous [SC], intramuscular [IM], intravenous [IV], per os [PO], orotransmucosal [OTM], transdermal patch [TDP], transdermal spot on [TDSO]).**

   ☐ Before or at the time of premedication

   ☐ After induction of anaesthesia or during surgery

   ☐ At the end of anaesthesia during the recovery period

3. **Do you routinely send dogs that have undergone <u>elective</u> ovariohysterectomy home with ongoing opioid therapy (please circle)?**

   YES NO

   i. **If YES, how do you typically prescribe this opioid: (please tick one):**

   ☐ Oral transmucosal ☐ Transdermal spot on

   ☐ Oral tablets ☐ Systemic (injection)

   ☐ Transdermal patch

   ii. **If YES, for how many days do you continue therapy in the home environment? (number of days):** ☐

4. **When considering *which* opioid to give in the peri-operative period to dogs how important (on a scale of 0 [not important] to 3 [very important]) do you rate the following factors (please circle)?**

	Not Important			Very Important
Analgesic efficacy	0	1	2	3
How painful the animal is before surgery	0	1	2	3
How painful the animal is likely to be during or after surgery	0	1	2	3
Data sheet (SPC) indications	0	1	2	3
Reported safety (side effects & tolerance)	0	1	2	3
Duration of action	0	1	2	3
Cost	0	1	2	3
Record keeping / storage requirements	0	1	2	3
Experience of using the opioid	0	1	2	3
Ease of continuing therapy after discharge (e.g. formulation)	0	1	2	3

5. **List the factors that you take into consideration when deciding which route (e.g. SC, IV, IM, transdermal) to administer an opioid peri-operatively to dogs? (please list all that apply)**

C: Use of local anaesthetic techniques

1. **Do you routinely use any of the following local anaesthetic (LA) techniques in dogs (please circle)?**

Maxillary or mandibular nerve block for dental procedures	YES	NO
Infiltration of local anaesthetic around teeth for dental procedures	YES	NO
Infiltration of local anaesthetic around skin incisions	YES	NO
Intratesticular local anaesthetic for castration	YES	NO
Epidural anaesthesia or analgesia for hind limb procedures	YES	NO
Brachial plexus block for procedures distal to elbow	YES	NO
Infiltration of local anaesthetic into surgical wounds	YES	NO
Use of infiltration catheters placed into surgical wounds	YES	NO
Mid humeral block (RUMM block) for procedures distal to the elbow	YES	NO

D: Use of adjunctive analgesic drugs

1. **Have you *ever* used the following drugs administered by continuous intravenous infusion (CRI) specifically for the provision of peri-operative analgesia in dogs?**

			If **yes** please give typical dose rate or range (mg or µg / kg/ hour or min)
Ketamine	YES	NO	
Lidocaine	YES	NO	
Medetomidine	YES	NO	
Dexmedetomidine	YES	NO	
Morphine / lidocaine / ketamine (MLK) mix	YES	NO	
Fentanyl / lidocaine / ketamine (FLK) mix	YES	NO	

2. **When using any of these drugs by CRI do you typically administer using controlled infusion apparatus (e.g. syringe driver or pump; please circle)?**

YES NO

E. The following question relates to your use of drugs for providing analgesia to dogs

Listed below is a selection of surgical procedures and medical conditions that you may or may not consider require analgesic administration and are commonly encountered in dogs. Please indicate **which drugs** you would administer for the listed conditions and procedures, and for **how long** you would administer them for after surgery

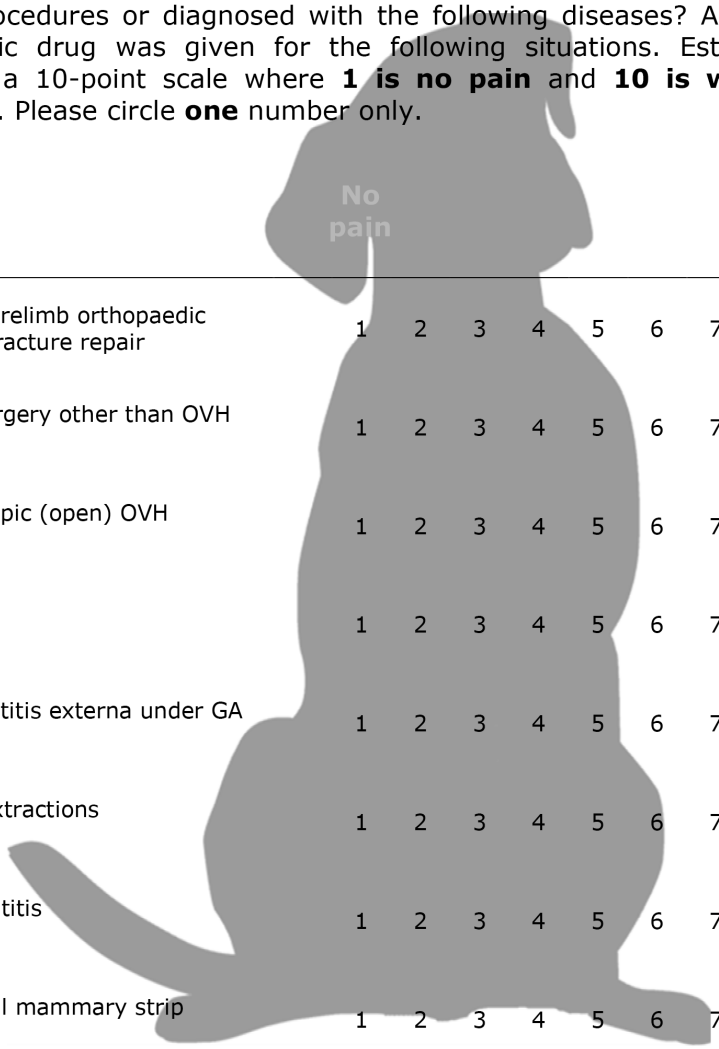
Procedure or condition	What type(s) of analgesic drug or technique would you typically use for this condition? Tick/ complete as many as apply					If you would use an opioid for premedication which opioid do you use?	If you would use additional opioids intra-operatively		If you would give opioids post-operatively			If used, for how long would you continue each class of analgesic drug after the end of surgery (please tick)					
	Opioid	NSAID	Local anaesthetic technique (please specify technique e.g. local block, epidural)	Adjunctive analgesics (please specify drug)	Tramadol		Which opioid do you use?	How would you give this opioid? CRI, IV IM, SC TDP, TDSO	Which opioid would you use?	How would you give this opioid? CRI, IV, IM, SC, TDP, TDSO	How frequently would you administer this opioid?	Drug class	< 24 hours	24-48 hours	48-72 hours	3-7 days	>7 days
Forelimb or hindlimb orthopaedic surgery (e.g. fracture repair)												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Abdominal surgery other than OVH												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
OVH (non laparoscopic)												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Castration												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Ear flush for otitis externa under GA												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Dental with extractions												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Acute pancreatitis												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Uni or bilateral mammary strip (mastectomy)												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					

CRI = continuous rate infusion; TDP = transdermal patch; TDSO = transdermal spot on; OVH = ovariohysterectomy

Part IV

The following section relates to pain assessment in adult dogs.

In your opinion how severe would the pain be in adult dogs undergoing the following procedures or diagnosed with the following diseases? Assume that **NO** analgesic drug was given for the following situations. Estimate pain severity on a 10-point scale where **1 is no pain** and **10 is worst pain imaginable**. Please circle **one** number only.



	No pain									Worst pain
Hindlimb or forelimb orthopaedic surgery e.g. fracture repair	1	2	3	4	5	6	7	8	9	10
Abdominal surgery other than OVH	1	2	3	4	5	6	7	8	9	10
Non laparoscopic (open) OVH	1	2	3	4	5	6	7	8	9	10
Castration	1	2	3	4	5	6	7	8	9	10
Ear flush for otitis externa under GA	1	2	3	4	5	6	7	8	9	10
Dental with extractions	1	2	3	4	5	6	7	8	9	10
Acute Pancreatitis	1	2	3	4	5	6	7	8	9	10
Uni or bilateral mammary strip (mastectomy)	1	2	3	4	5	6	7	8	9	10

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Part V

The following questions relate to your use of analgesics in the peri-operative period in CATS:

A: Administration of NSAIDs

1. **Do you typically administer NSAIDs in the peri-operative period to healthy cats undergoing routine procedures (e.g. lump removal, ovariohysterectomy, castration)?**

   YES NO

2. **If YES, in the peri-operative period, in an individual healthy cat when do you typically administer the NSAID? (please tick one and state route of administration – subcutaneous [SC], intramuscular [IM], intravenous [IV], per os [PO]).**

Before or at the time of premedication		At the end of anaesthesia during the recovery period	
After induction of anaesthesia or during surgery		Other (please specify time & route)	

3. **Do you routinely send cats that have undergone <u>elective</u> ovariohysterectomy home with ongoing NSAID oral therapy (please circle)?**

   YES NO

   i. **If YES, for how many days do you typically continue oral NSAID therapy? (number of days):**

4. **When considering *which* NSAID to give in the peri-operative period to cats how important (on a scale of 0 [not important] to 3 [very important]) do you rate the following factors (please circle)?**

	Not Important			Very Important
Analgesic efficacy	0	1	2	3
Availability of an injectable preparation	0	1	2	3
Data sheet (SPC) indications	0	1	2	3
COX 1/ COX 2 selectivity	0	1	2	3
Tissue selectivity	0	1	2	3
Reported safety (side effects & tolerance)	0	1	2	3
Continuation of same NSAID if patient is on long term therapy	0	1	2	3
Available product literature / information	0	1	2	3
Cost	0	1	2	3
Practice purchasing policy	0	1	2	3
Relationship with company representative	0	1	2	3
Ease of continuing therapy after discharge (e.g. formulation, palatability)	0	1	2	3

B: Administration of opioids

1. **Do you typically administer opioids in the peri-operative period to healthy cats undergoing routine procedures (e.g. lump removal, ovariohysterectomy, castration)?**

   YES NO

2. **If YES, in the peri-operative period, in an individual healthy cat when do you typically administer the opioid? (please tick ALL that apply and state route of administration – subcutaneous [SC], intramuscular [IM], intravenous [IV], per os [PO], orotransmucosal [OTM], transdermal patch [TDP].**

   ☐ Before or at the time of premedication

   ☐ After induction of anaesthesia or during surgery

   ☐ At the end of anaesthesia during the recovery period

3. **Do you routinely send cats that have undergone <u>elective</u> ovariohysterectomy home with ongoing opioid therapy (please circle)?**

   YES NO

   i. **If YES, how do you typically prescribe this opioid: (please tick one):**

   ☐ Oral transmucosal ☐ Transdermal patch

   ☐ Oral tablets ☐ Systemic (injection)

   ii. **If YES, for how many days do you continue therapy in the home environment? (number of days):** ☐

4. **When considering *which* opioid to give in the peri-operative period to cats how important (on a scale of 0 [not important] to 3 [very important]) do you rate the following factors (please circle)?**

	Not Important			Very Important
Analgesic efficacy	0	1	2	3
How painful the animal is before surgery	0	1	2	3
How painful the animal is likely to be during or after surgery	0	1	2	3
Data sheet (SPC) indications	0	1	2	3
Reported safety (side effects & tolerance)	0	1	2	3
Duration of action	0	1	2	3
Cost	0	1	2	3
Record keeping / storage requirements	0	1	2	3
Experience of using the opioid	0	1	2	3
Ease of continuing therapy after discharge (e.g. formulation)	0	1	2	3

5. **List the factors that you take into consideration when deciding which route (e.g. SC, IV, IM, transdermal) to administer an opioid peri-operatively to cats? (please list all that apply)**

C: Use of local anaesthetic techniques

1. **Do you routinely use any of the following local anaesthetic (LA) techniques in cats (please circle)?**

Maxillary or mandibular nerve block for dental procedures	YES	NO
Infiltration of local anaesthetic around teeth for dental procedures	YES	NO
Infiltration of local anaesthetic around skin incisions	YES	NO
Intratesticular local anaesthetic for castration	YES	NO
Epidural anaesthesia or analgesia for hind limb procedures	YES	NO
Brachial plexus block for procedures distal to elbow	YES	NO
Infiltration of local anaesthetic into surgical wounds	YES	NO
Use of infiltration catheters placed into surgical wounds	YES	NO
Mid humeral block (RUMM block) for procedures distal to the elbow	YES	NO

D: Use of adjunctive analgesic drugs

1. **Have you *ever* used the following drugs administered by continuous intravenous infusion (CRI) specifically for the provision of peri-operative analgesia in cats?**

			If **yes** please give typical dose rate or range (mg or µg / kg/ hour or min)
Ketamine	YES	NO	
Lidocaine	YES	NO	
Medetomidine	YES	NO	
Dexmedetomidine	YES	NO	
Morphine / lidocaine / ketamine (MLK) mix	YES	NO	
Fentanyl / lidocaine / ketamine (FLK) mix	YES	NO	

2. **When using any of these drugs by CRI do you typically administer using controlled infusion apparatus (e.g. syringe driver or pump; please circle)?**

YES NO

E. The following question relates to your use of drugs for providing analgesia to cats

Listed below is a selection of surgical procedures and medical conditions that you may or may not consider require analgesic administration and are commonly encountered in cats. Please indicate **which drugs** you would administer for the listed conditions and procedures, and for **how long** you would administer them for after surgery

Procedure or condition	What type(s) of analgesic drug or technique would you typically use for this condition? Tick/ complete as many as apply					If you would use an opioid for premedication which opioid do you use?	If you would use additional opioids intra-operatively		If you would give opioids post-operatively			If used, for how long would you continue each class of analgesic drug after the end of surgery (please tick)					
	Opioid	NSAID	Local anaesthetic technique (please specify technique e.g. local block, epidural)	Adjunctive analgesics (please specify drug)	Tramadol		Which opioid do you use?	How would you give this opioid? CRI, IV IM, SC TDP	Which opioid would you use?	How would you give this opioid? CRI, IV, IM, SC, TDP	How frequently would you administer this opioid?	Drug class	< 24 hours	24-48 hours	48-72 hours	3-7 days	>7 days
Forelimb or hindlimb orthopaedic surgery (e.g. fracture repair)												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Ruptured diaphragm repair												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Abdominal surgery other than OVH												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
OVH												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Castration												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Dental with extractions												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Acute pancreatitis												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					
Relief of urethral obstruction												NSAID					
												Opioid					
												Adjunctive					
												Tramadol					

CRI = continuous rate infusion; TDP = transdermal patch; TDSO = transdermal spot on; OVH = ovariohysterectomy

Part VI

The following section relates to pain assessment in adult cats.

In your opinion how severe would the pain be in adult cats undergoing the following procedures or diagnosed with the following diseases? Assume that **NO** analgesic drug was given for the following situations. Estimate pain severity on a 10-point scale where **1 is no pain** and **10 is worst pain imaginable**. Please circle **one** number only.

	No pain									Worst pain
Hindlimb or forelimb orthopaedic surgery e.g. fracture repair	1	2	3	4	5	6	7	8	9	10
Ruptured diaphragm repair	1	2	3	4	5	6	7	8	9	10
Abdominal surgery other than OVH	1	2	3	4	5	6	7	8	9	10
OVH	1	2	3	4	5	6	7	8	9	10
Castration	1	2	3	4	5	6	7	8	9	10
Dental with extractions	1	2	3	4	5	6	7	8	9	10
Acute Pancreatitis	1	2	3	4	5	6	7	8	9	10
Relief of urethral obstruction	1	2	3	4	5	6	7	8	9	10

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Part VII

The following questions relate to both cats and dogs

A. Pain assessment

1. **Does the practice routinely use a pain assessment tool in the peri-operative period in dogs (please circle)?**

   YES NO

   If yes, which tool is routinely used?

2. **Does the practice routinely use a pain assessment tool in the peri-operative period in cats (please circle)?**

   YES NO

   If yes, which tool is routinely used?

3. **Who in the practice is responsible for pain assessment in the perioperative period (please circle)?**

   VET

   NURSE

   VET & NURSE

   OTHER (please specify)

4. **Does the practice routinely instruct owners to assess pain in their dog or cat after discharge (please circle)?**

   YES NO

5. **If YES, how do you do this? PLEASE SPECIFY (e.g. pain assessment protocol, scoring system)**

Part VIII

These questions relate to your own knowledge of peri-operative analgesia in dogs and cats

1. **Do you consider your knowledge in the area of peri-operative analgesia provision in small animals to be adequate (please circle)?**

   YES NO

2. **What is your preferred way of updating your knowledge? (please tick all that apply)**

- ☐ Day CPD meeting (e.g. via BSAVA or a commercial CPD provider)
- ☐ Webinar
- ☐ Evening CPD meeting
- ☐ In house CPD (e.g. delivered by other practice members or a drug company)
- ☐ Reading non-peer reviewed articles (e.g. Vet Times)
- ☐ Reading peer reviewed articles e,g In Practice, UK Vet, Vet Rec, JAVMA
- ☐ On line forums e.g. VIN, vetsurgeon.org
- ☐ Web-based learning tools that can be done independently at a convenient time

Thank you for the taking the time to complete this questionnaire

Please return in the envelope provided

2.3 Defining adverse events related to prescription of non-steroidal anti-inflammatory drugs

Non-steroidal anti–inflammatory drugs are effective antipyretics and analgesics in acute and chronic inflammatory pathologies (Slingsby & Waterman-Pearson, 2000; Lascelles et al., 2001; Lascelles et al., 1998; Moreau et al., 2003). In Paper 2 these drugs were shown to be the class of perioperative analgesics most commonly prescribed to dogs and cats (Hunt et al., 2015), but they may be associated with a range of adverse events. An adverse event is defined by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) as "*any observation in animals, whether or not considered to be product-related, that is unfavourable and unintended and that occurs after any use of Veterinary Medicinal Product (VMP) (off-label and on-label uses)*". Discussion of the risks versus benefits of NSAID prescription may be hampered by a lack of published data on the likelihood of adverse events in animals.

Non-steroidal anti-inflammatory drugs act by inhibiting cyclo-oxygenase enzymes, therefore reducing the formation of prostaglandins from arachidonic acid located in cell membranes. Cyclo-oxygenase (COX) exists in at least two forms (Vane et al., 1998), COX-1 mainly functioning in a constitutive homeostatic role and COX-2 an inducible form responsible for increasing the production of prostaglandins in inflammatory states. This knowledge has led to the development of COX-2 selective and specific drugs in an attempt to reduce the side effects associated with the reduction of homeostatic prostaglandins in organs such as the stomach and kidney.

A variety of NSAID molecules have marketing authorisation for treatment of pain and inflammation in dogs and cats, ranging from COX-1 selective drugs such as ketoprofen, through COX-2 preferential drugs such as meloxicam and carprofen (Streppa et al., 2002) to COX-2 selective drugs such as firocoxib, robenacoxib, mavacoxib and cimicoxib (McCann et al., 2004; King et al., 2010; Carmichael, 2011). Whilst theory suggests that improved safety would be associated with the use of COX-2 selective drugs, there is limited evidence of the frequency and severity of adverse events (AEs) related to NSAIDs with marketing authorisation for long term use in the target population of cats and dogs, and none which documents improved safety resulting from the use of COX-2 selective drugs rather than COX-2 preferential drugs. In man, COX-2 selective drugs were shown to be associated with a lower risk of gastrointestinal adverse events compared with non-selective NSAIDs (Moore, A. et al., 2013), although increased risk of cardiovascular adverse events associated with the COX-2 selective rofecoxib (Baron et al., 2008) led to the withdrawal of this drug from the market. Subsequent work has identified that most NSAIDs are associated with an increased risk of cardiovascular events in man (Barcella, et al., 2019).

A meta-analysis of 14 studies assessing treatment of osteoarthritis in dogs of more than one month's duration, performed by Innes et al., (2010), reported calculated experimental (adverse) event rates ranging from 0 to 0.31, though the failure of many studies to include a placebo group limited the ability to estimate 'numbers needed to harm'. The rate of adverse events did not appear to increase with increasing duration of treatment, however the authors concluded that "*robust data on the safety of long-term NSAID use are lacking in*

large numbers of dogs". Only one AE which was considered serious was reported in the trials assessed, which was an episode of toxic hepatitis in a Labrador.

Data from field trials of meloxicam at authorised doses (Boehringer Ingelheim Vetmedica, 2003) indicate a reported incidence of 28% for vomiting in 109 dogs during a two week treatment period, compared to an incidence in the control group of 13% (n=115) (p = 0.005), though the incidence of diarrhoea/soft stools was not different between groups (14% and 10%). Over a four week study period adverse gastrointestinal side effects were noted in 4 out of 38 dogs (11%) administered meloxicam at authorised doses (Doig, 2000). Lower incidences of vomiting in dogs treated with carprofen over a two week period (Pfizer, 2002) (3.1% of 129) or placebo (3.8% of 132) were reported, incidence of diarrhoea in this study was also low (3.1 and 4.5 %).

Incidence of vomiting over a 30 day treatment period with firocoxib was 4% in 128 dogs, contrasted with the positive control etodolac (7% in 121 dogs) (Turner, 2004).

Autefage et al., (2011) reported treatment of 39 dogs, with clinical and radiographic signs of osteoarthritis, with 5mg kg^{-1} firocoxib over a period of 360 days. One dog was withdrawn from the trial due to diarrhoea, one dog suffered a fatal perforating gastric ulcer a few days after accidentally being treated with a double dose of firocoxib, and two dogs were withdrawn from the study due to elevations in serum creatinine.

In a non-inferiority study of robenacoxib and carprofen in a population of osteoarthritic dogs the most frequent adverse events reported were diarrhoea or vomiting (robenacoxib group 23%, carprofen group 24%; no significant difference in frequency between groups)

which were mostly benign and transient. Blood in the faeces was noted in one of 125 dogs receiving robenacoxib and two of 63 dogs receiving carprofen, again no significant difference in frequency of this AE was identified between groups (Reymond et al., 2012).

These varying estimates of incidence are undoubtedly related to the relatively small (compared to the number of prescriptions for these drugs issued) sample sizes. However as these studies, combined with data from case reports (Reed, 2002; Duerr et al., 2004; Enberg et al., 2006) show, gastrointestinal side effects are not uncommonly encountered in dogs treated with NSAIDs.

In an experimental toxicological study involving three male and three female dogs treated with intravenous meloxicam 0.2 mg kg^{-1} (double the recommended maintenance dose) for 3 days (Boehringer Ingelheim Vetmedica, 2003), one dog vomited on the third day of treatment. At post-mortem examination all three female dogs exhibited areas of congestion, inflammation and haemorrhage in the colon, ileum, and caecum. Whilst the sample size is too small to draw conclusions, it suggests the potential for sex related differences in the occurrence of NSAID related AEs. Evidence from medical literature also suggests that female patients have an increased risk of serious gastrointestinal AEs (Neutel et al., 1999).

In addition to gastrointestinal adverse effects, other reported potential consequences associated with the use of NSAIDs in dogs at recommended doses include hepatocellular necrosis (Nakagawa et al., 2005), toxic hepatitis (Moreau et al., 2003; MacPhail et al., 1998),

prolongation of bleeding times (Luna et al., 2007), leucocytosis, fever, elevations in gamma glutamyl transferase, blood urea nitrogen and total calcium (Boehringer Ingelheim Vetmedica, 2003). At doses of meloxicam five times higher than recommended for three days two out of three female dogs developed acute renal failure accompanied by clinically significant increases in urine protein concentration. Dogs treated with five times the recommended dose also displayed increases in alkaline phosphatase (ALP) and serum calcium, and decreased serum total protein (Boehringer Ingelheim Vetmedica, 2003). In a study of firocoxib administered at five times the recommended dosage for 6 months, transient elevations in white blood cell and platelet counts and decreases in albumin were reported, which had returned to normal levels by the completion of the study (Turner, 2004). In that study, thalamic vacuolation was reported to be more severe in dogs treated with five times the recommended dosage of firocoxib compared to control dogs that received placebo. Although mean ALP concentrations remained within normal ranges for all groups, concentrations were significantly higher in dogs treated with 3x and 5x the recommended dosage compared to controls (Turner, 2004). In dogs treated with 5 to 10 times the recommended doses of carprofen for 6 weeks, hypoalbuminaemia was reported in some dogs (Pfizer, 2002).

Data on adverse events during treatment of naturally occurring osteoarthritis in cats are similarly sparse, though there is some evidence that cats experiencing AEs related to meloxicam were treated for longer durations than cats not experiencing AEs (Charlton et al., 2013). In that study 4 out of 22 (18%) cats experienced adverse events related to daily treatment with a mean dose of 0.027 mg kg^{-1} meloxicam, after a median duration of treatment of 448 days and, in 3 cats, vomiting, excessive salivation and diarrhoea

necessitated stopping treatment. Daily doses of meloxicam from 0.01-0.03 mg kg^{-1} were administered to 40 osteoarthritic cats for a mean of 5.8 months (Gunew et al., 2008). Gastrointestinal upset related to meloxicam administration was reported in 2 out of 46 cats in that study.

Toxicity studies of robenacoxib in cats cite soft stools as the most frequent adverse event related to treatment, occurring in up to a third of animals treated with 2-10 times the recommended dose of robenacoxib, but found that this was not different to the incidence in placebo treated animals (King et al., 2011).

When used at lower than authorised dose rates in a cohort of older cats with osteoarthritis (Gowan et al., 2011) meloxicam did not appear to accelerate the progression of pre-existing renal disease, and did not appear to increase the incidence of renal disease in a cohort of cats without renal disease at the beginning of the study.

During 35 days of treatment with robenacoxib in cats at 3 and 5 times the recommended dosage transient decreases in mean cell haemoglobin concentration compared to placebo were identified after 14 days, but there was no difference to placebo after 35 days (King et al., 2011). In this study minor decreases in activity of alanine aminotransferase and aspartate aminotransferase were also reported in the 5 x dose group. A report of an injection site sarcoma ascribed to injection of meloxicam has been published (Munday et al., 2011).

Experimentally, development of NSAID induced duodenal ulceration in cats has been shown to be increased by insoluble dietary fibre and decreased by administration of 5-lipoxygenase inhibitors, leukotriene antagonists and cholinergic antagonists (Satoh et al., 2009).

In human population studies increased age, increased dose of NSAID, and a short term (less than 1 month) history of taking NSAIDs were associated with increased risk of hospitalisation for gastrointestinal bleeding (Lanza et al., 2009). Increased susceptibility to NSAID induced GI ulceration has also been demonstrated with age in rats (Hong et al., 2012).

The government body responsible for collating details of adverse events related to veterinary medicinal products, the Veterinary Medicines Directorate (VMD), publishes an annual summary of information[4], however no previous work has endeavoured to estimate the frequency of adverse events related to administration of veterinary medicinal products, nor to relate risk of adverse events to demographic variables such as age and sex. To this end, an analysis of the relative frequencies of adverse events, associated with different NSAID molecules with marketing authorisation for use in dogs and cats and reported to the VMD, was undertaken.

[4] https://www.gov.uk/government/collections/veterinary-medicines-pharmacovigilance-annual-review-reports accessed 8th March 2019

2.3.1 Paper 3. Hunt, J.R., Dean, R.S., Davis, G.N.D., Murrell, J.C., 2015. An analysis of the relative frequencies of reported adverse events associated with NSAID administration in dogs and cats in the United Kingdom. The Veterinary Journal 206, 183–190.

Randomised controlled clinical studies are designed to possess adequate statistical power to investigate the efficacy of a drug, but will be underpowered to investigate the incidence of rarely occurring AEs (Tramèr et al., 2000). Additionally, reporting of AEs within clinical trials may not always be complete (Edwards et al., 1999). Given the documented widespread prescription of NSAIDs and lack of evidence relating to AEs discussed above, there was a clear need to better understand the likelihood of drug associated adverse events. Following a literature review (summarised above), we hypothesised that the overall rates of NSAID related side effects in target populations of cats and dogs would be less than 10%, and that COX-2 selective drugs would be associated with a lower incidence of gastrointestinal adverse events than COX-2 preferential and COX-1 preferential drugs. We also hypothesised that increasing age would be associated with an increased risk of gastrointestinal adverse events.

The original aims of the study were

1. To document the incidence of adverse events associated with the use of carprofen, meloxicam, firocoxib, robenacoxib, mavacoxib and cimicoxib (in dogs) and meloxicam, carprofen and robenacoxib (cats) as reported to the Veterinary Medicines Directorate by drug companies, veterinary surgeons or animal owners.

2. To determine any differences in incidence of adverse events between the various NSAID molecules.

3. To determine (by a prospective trial incorporating an owner questionnaire) the incidence of adverse events in a first opinion population of animals prescribed an NSAID molecule for osteoarthritis.

4. To determine any differences in incidence of reported adverse events compared to the incidence expected as a result of the prospective trial.

5. Further analyse the data to determine if any breed or age associations are evident.

Our initial approach was to discuss the proposed project with the co-ordinator for the National Office of Animal Health (NOAH), an industry body which represents the majority of the pharmaceutical companies producing veterinary medicines in the United Kingdom. The result of this approach was that a number of manufacturers expressed concerns regarding the disclosure of data related to the size of their market, and any skewing of data related to frequency of adverse events (e.g. more recent marketing authorisation). The recommendation from NOAH was to approach the VMD so that data would be provided in a standardised format for all manufacturers of veterinary medicines. Following writing to the VMD to request information, we were invited to a meeting to discuss the project. Following our presentation, which highlighted the aims of determining valid estimates for the incidence of NSAID related AEs in first opinion veterinary medicine, and identifying patient

factors associated with increased risk of AEs, the VMD agreed to provide data in a limited form, to avoid any potential contravention of commercial sensitivity. Cumulative frequencies of AEs, since the date of marketing authorisation, were provided for each drug molecule. The VMD calculated the frequencies by dividing the number of reports of AEs by the number of doses of each product used. To calculate the number of doses, the volumes of products sold were divided by the average volume per dose, assuming an average dog weight of 20kg and cat weight of 5kg. Adverse events were classified according to Veterinary Medicinal Dictionary for Drug Regulatory Authorities (VeDDRA) terms and provided in a spreadsheet file.

Unfortunately, the time required to obtain the pharmacovigilance information precluded conducting the intended prospective study to document the incidence of AEs associated with new NSAID prescriptions in first opinion practice. Demographic data associated with AE reports was also not made available, therefore no descriptive analysis of the affected animals was possible.

From the information provided we were able to identify the ten most commonly reported AEs for each molecule, compare the reported frequencies of AEs between coxibs and non-coxibs administered orally to dogs, compare the reported frequencies of AEs between oral and injectable formulations across both species, analyse the correlation between frequency of reported AEs and time since marketing authorisation, and compare the reported frequency of AEs associated with oral NSAID administration between cats and dogs.

We identified that there were no significant differences in reported frequency of AEs associated with oral NSAID administration between cats and dogs. There was a significant negative correlation between reported frequency of AEs and time since marketing

authorisation. Reported frequencies of AEs were higher in association with coxibs compared with non-coxibs in dogs, and injectable compared with oral formulations in both dogs and cats. We also identified that convulsions were reported as an AE associated with NSAIDs in dogs and cats.

Without knowing the size of the population at risk, it was not possible to draw any conclusions regarding the incidence of AEs related to NSAID prescription, therefore the relative frequency of reports, as a fraction of the estimated number of doses sold (but not administered) was used as a measure to compare the scale of AEs associated with different molecules and formulations. Unfortunately, given the assumptions that were required to be made to calculate the relative frequencies of AEs, these figures are in no way comparable to incidences of AEs reported in previous studies, therefore accurate estimates for the incidence of NSAID related AEs remain to be determined.

Although the data were challenging to interpret due to a number of potential confounding factors, including the time elapsed since marketing authorisation, severity of the adverse event, and clinical circumstances likely to be associated with differing routes of administration, our analysis provides a basis for further studies. It is possible that data collected through initiatives such as VetCompass™ may provide a more valid estimate of the incidence of AEs which precipitate veterinary examination, compared with the passive surveillance system that was used to generate data for the present study. Large scale prospective studies to actively document AEs associated with new NSAID prescriptions and identify demographic risk factors for AEs would also be expected to generate useful data. The finding that convulsions were reported as an adverse event associated with most of the

NSAID molecules considered was unexpected. Clearly the data are insufficient to demonstrate causality, and these adverse events may be related to the underlying medical condition of the patient rather than to drug administration, but this study highlights the value of examining pharmacovigilance data for rare or unexpected adverse events.

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An analysis of the relative frequencies of reported adverse events associated with NSAID administration in dogs and cats in the United Kingdom

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ABSTRACT

This study aimed to analyse UK pharmacovigilance data to quantify adverse events (AEs) associated with the non-steroidal anti-inflammatory drug (NSAID) molecules found in veterinary medicines authorised for use in dogs and cats. It was hypothesised that the frequency of AEs would be lower when associated with cyclo-oxygenase-2 selective (coxib), compared to non-selective (non-coxib) NSAIDs. The UK Veterinary Medicines Directorate (VMD) supplied frequencies of AEs derived from Periodic Safety Update Reports subdivided by formulation and species for each NSAID molecule.

Frequencies of AEs were similar between species. The five most reported AEs were emesis, death, anorexia, lethargy, and diarrhoea. Reported frequency of emesis, renal insufficiency and death was higher with injectable compared to oral NSAIDs ($P = 0.043$). Reported frequency of emesis, lethargy and death was higher with coxib, compared to non-coxib NSAIDs ($P = 0.029$). Median (range) interval since authorisation was shorter for coxibs at 5 (2.5–9) years compared to non-coxibs at 15 (12–25) years. A negative correlation between time elapsed since authorisation and the frequency of AEs was identified ($r_s = -0.11$ to -0.94). Higher frequency of reported AEs with injectable NSAIDs may be related to perioperative administration. The AE frequency associated with coxib and non-coxib NSAIDs may be confounded by changes in reporting habits over time.

This study highlights the value of interrogating passive surveillance data to identify low frequency AEs and the need to facilitate improvement in recording and collecting AEs in small animal practice.

Introduction

Prescription of non-steroidal anti-inflammatory drugs (NSAIDs) to dogs and cats is commonplace for perioperative analgesia (Farnworth et al., 2014; Hunt et al., 2015) and management of painful inflammatory conditions such as osteoarthritis (Sanderson et al., 2009; Sparkes et al., 2010). Concern exists amongst veterinary surgeons (Capner et al., 1999; Hugonnard et al., 2004) and pet owners[1,2] about potential adverse effects (AEs) of NSAIDs in pet animals.

An AE is defined by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) as '*any observation in animals, whether or not considered to be product-related, that is unfavourable and unintended and that occurs after use of a Veterinary Medicinal Product (VMP) (off-label and on-label uses)*'. Most NSAID AEs are mild and self-limiting (Forsyth et al., 1998; Leong and Chan, 2006), although serious AEs, defined by the VICH as '*an event which results in death, is life-threatening, results in persistent or significant disability/incapacity, or a congenital anomaly or birth defect*', coincident with NSAID administration are reported (Duerr, 2004; Enberg et al., 2006).

It has been proposed that selective cyclo-oxygenase 2 (COX-2) inhibitors decrease NSAID AEs (Simmons et al., 2004). Increased tolerability of COX-2 selective (coxib), compared to non-selective (non-coxib), NSAIDs in dogs (Wooten et al., 2009; Reymond et al., 2012) or cats (Kamata et al., 2012; Sano et al., 2012) has, however, not been reported.

Recently, two reviews of NSAID AEs in dogs have been published. Monteiro-Steagall et al. (2013) concluded that '*most studies were not appropriately designed to determine the safety of NSAIDs, and involved a healthy non-geriatric population of research dogs*', whilst

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[1] See: http://www.dailymail.co.uk/news/article-2127028/Could-drug-cost-beloved-pet-life-kill-YOUR-dog-Vet-raises-alarm-arthritis-pill-prescribed-millions-animals.html. (accessed 27 February 2015).

[2] See: http://ths.gardenweb.com/discussions/2499315/i-suspect-metacam-side-effects-other-options. (accessed 27 February 2015).

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Innes et al. (2010) commented that '*robust data on the safety of long-term NSAID use are lacking in large numbers of dogs*'. No published studies address the frequency of NSAID AEs in cats. Randomised controlled clinical studies are powered to investigate the efficacy of a drug, and are unlikely to generate valid estimates of the incidence of rare AEs (Tramèr et al., 2000). Moreover, reporting of AEs within clinical trials may not always be complete (Edwards et al., 1999).

The UK Veterinary Medicines Directorate (VMD) administers a passive AE surveillance programme and summary reports are published regularly (Dyer et al., 2011, 2012). Marketing Authorisation Holders (MAHs) submit Periodic Safety Update Reports (PSURs) to the VMD and these incorporate instances of non-serious AEs that have been reported to the MAH, but may not have generated an AE report to the VMD.

Under-reporting of AEs is likely. Dean et al. (2013) found that the number of reports of feline injection site sarcomas to the VMD in 2007 was fewer than the number identified during a period of active surveillance for the condition. However, the data collated by the VMD represent the most comprehensive record of AEs available for NSAIDs, and was therefore considered appropriate for this analysis. It is recognised that whilst passive surveillance schemes are subject to reporting biases (Wallenstein and Fife, 2001; Hartnell and Wilson, 2004), their value lies in the comparison of the frequency of AEs associated with products which would be expected to be subject to similar biases; and in the identification of 'signal' data of low frequency or unanticipated AEs (Williams, 2012).

The aim of this study was to investigate the reported relative frequencies of adverse events (AEs) associated with NSAIDs in dogs and cats. We hypothesised that coxibs would be associated with fewer gastrointestinal AEs, compared to non-coxibs.

Materials and methods

Terminology

We considered that the NSAIDs available comprised individual *molecules* (e.g. carprofen, meloxicam, robenacoxib), which were formulated by MAHs into authorised *preparations*, which could be injectable or oral *formulations*.

The VMD supplied cumulative frequencies of AEs, calculated since the date of marketing authorisation (MA) of each molecule (Table 1) and classified in a spreadsheet file (Excel 2007, Microsoft) according to terms for clinical signs given in the European Medicines Agency's Veterinary Dictionary for Drug Related Affairs (VeDDRA).[3] Data were supplied regarding reported AEs in dogs and cats for the molecules carprofen, cimicoxib, cinchophen/prednisolone, firocoxib, ketoprofen, mavacoxib, meloxicam, paracetamol/codeine, robenacoxib, and tepoxalin. Data for each molecule were made up of a composite of data from each authorised preparation.

Table 1
Length of time since granting of marketing authorisation (years) and proportion of product assumed prescribed to cats and dogs by the Marketing Authorisation Holders and Veterinary Medicines Directorate for the purpose of calculating the frequency of adverse events.

Active	Date of MA	Years since MA	Proportion administered to dog (%)	Proportion administered to cat (%)
Carprofen injection	15/03/93	20	80	20
Carprofen oral	17/03/93	20	100	0
Cimicoxib	18/02/11	2.5	100	0
Cinchophen	01/12/92	21	100	0
Firocoxib	13/09/04	9	100	0
Ketoprofen injection	18/05/92	21	60	40
Ketoprofen oral	29/04/92	21	5 mg 75 20 mg 100	5 mg 25 20 mg 0
Mavacoxib	09/09/08	5	100	0
Meloxicam injection	24/06/96	17	75	25
Meloxicam oral cat	20/04/07	8	0	100
Meloxicam oral dog	28/09/92	21	100	0
Paracetamol	15/04/93	20	100	0
Robenacoxib injection	16/12/08	5	75	25
Robenacoxib oral cat	16/12/08	5	0	100
Robenacoxib oral dog	16/12/08	5	100	0
Tepoxalin	13/03/01	12	100	0

MA, marketing authorisation.

Table 2
Products and time periods for which periodic safety update report data were not available to the Veterinary Medicines Directorate.

Product	Active	Dates during which PSUR data were unavailable
Zenecarp 5% injection	Carprofen	October 1997–January 2000
Ketofen 1% solution for injection	Ketoprofen	September 1993–December 1995
Metacam 1.5 mg/mL oral suspension for dogs	Meloxicam	January 2002–June 2002
Metacam 5 mg/mL solution for injection for dogs and cats	Meloxicam	January 2002–June 2002
Zubrin 30, 50, 100 and 200 mg oral lyophilisates for dogs	Tepoxalin	October 2001–September 2002

PSUR, periodic safety update report.

In molecules for which injectable and oral formulations were available (ketoprofen, carprofen, meloxicam and robenacoxib), frequencies for injectable and oral formulations were presented and analysed separately. Data for oral meloxicam and robenacoxib were presented separately for cats and dogs, as species-specific MAs permitted attribution of AEs to each species. Reports where the product was not assessed to have been likely responsible for the signs observed (i.e. causality coded N under the ABON system) (Woodward, 2005) were excluded.

The VMD estimated the number of doses of each NSAID preparation sold by dividing the total volume of product sales by the estimated average volume per dose. For tablets it was assumed one tablet constituted one dose. For injectable formulations and oral suspensions the average volume per dose was estimated, assuming average weights of 20 kg for dogs and 5 kg for cats. In the case of products authorised for use in more than one species, the number of doses sold was calculated using estimates of the proportion administered to each species, provided by MAHs (Table 1), as follows:

$$\text{Number of doses of product/formulation sold} = \frac{\text{Volume of product sold}}{\text{Average dose of product}}$$

The frequency of each reported AE for each NSAID was calculated by dividing the total number of reports of each VeDDRA term by the estimated number of doses sold since MA. Data unavailable for the NSAID preparations and periods are shown in Table 2.

$$\text{Frequency of reported AE for each molecule} = \frac{\text{Number of reports of AE}}{\text{Number of doses of product/formulation sold}}$$

For each molecule the 10 most common VeDDRA terms were identified (Table 3); these were predominantly AEs currently listed in the relevant NSAID Summary of Product Characteristics (SPCs) product literature, which accompany authorised NSAID products. The frequencies of the most commonly reported AEs were compared between different formulations, and classes, of NSAIDs. The duration of MA was calculated from the original date of MA of each molecule, obtained from the VMD website.[4] To provide a clinically useful metric, the reported frequency of each VeDDRA term was used to calculate the predicted number of AE reports per million doses of molecule sold.

Statistical methods

Prism 5 for Mac OSX (GraphPad) was used for statistical analysis. Data were assessed for normality using frequency histograms and Kolmogorov–Smirnov normality analysis. The data were not normally distributed; non-parametric methods were used throughout. Comparisons of the median frequency of an AE between groups or formulations (e.g. coxib vs. non-coxib; oral vs. injectable) were performed using Mann–Whitney *U*-tests. A Spearman non-parametric correlation was used to evaluate the correlation between the duration of MA and the frequency of each AE. Numerical

[3] See: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/07/WC500094802.pdf (accessed 20 July 2015).

[4] See: http://www.vmd.defra.gov.uk/ProductInformationDatabase/Default.aspx (accessed 18 June 2015).

Table 5
Predicted number of reports of adverse events per 1,000,000 administrations of drug, calculated from reported data to Veterinary Medicines Directorate. For mavacoxib the bracketed figure refers to a 1/30 of the per 1,000,000 administrations result in order to permit a comparison with drugs that are administered daily.

Product	Emesis	Death	Anorexia	Lethargy	Diarrhoea	Hepatopathy	Renal insufficiency	Haemorrhagic diarrhoea	Melaena	Haematemesis	Polydipsia	Convulsions	Dehydration
Carprofen oral	91	51	36	43	40	42	31	13	12	8	8	7	4
Carprofen injection	476	552	319	421	153	136	259	76	64	64	76	42	85
Cimicoxib	1971	282	751	469	845	NR	469	751	657	188	188	NR	NR
Cinchophen	12	6	5	3	5	1	NR	1	2	1	NR	1	NR
Firocoxib	170	113	74	83	65	65	52	52	39	30	9	13	17
Ketoprofen oral	48	60	24	36	NR	NR	12	NR	24	24	NR	24	12
Ketoprofen injection	458	595	183	183	NR	NR	320	NR	137	137	137	NR	92
Mavacoxib	51,849 (1728)	14,233 (474)	18,808 (627)	16,775 (559)	18,808 (627)	14,233 (474)	17,791 (593)	12,708 (424)	4575 (153)	4575 (153)	3558 (119)	1525 (51)	1017 (33.9)
Meloxicam oral cat	107	61	93	76	34	24	110	2	7	3	17	5	25
Meloxicam oral dog	109	67	48	54	43	22	36	38	19	24	7	10	9
Meloxicam injection	943	684	606	679	303	107	870	235	73	93	166	44	186
Paracetamol	18	18	18	NR	NR	18	NR	NR	18	NR	NR	NR	NR
Robenacoxib oral cat	400	267	267	267	NR	NR	133	NR	NR	NR	NR	NR	133
Robenacoxib oral dog	515	157	358	314	269	448	90	67	22	22	381	22	NR
Robenacoxib injection	884	1325	442	442	884	NR	442	NR	NR	442	NR	1325	NR
Tepoxalin	421	349	102	87	334	15	NR	160	203	116	NR	NR	NR

NR, not reported.

Table 6
Comparison of the predicted numbers of adverse event reports per million administrations of oral non-steroidal anti-inflammatory drugs in dogs and cats.

Adverse event	Predicted number of AE reports in dogs per 1,000,000 oral NSAID administrations	Predicted number of AE reports in cats per 1,000,000 oral NSAID administrations	*P*
Renal insufficiency	44 (31–469)	122 (110–133)	0.93
Emesis	170 (18–1970)	254 (107–400)	0.69
Anorexia	74 (24–751)	180 (93–267)	0.93
Lethargy	83 (36–469)	172 (76–267)	0.90
Death	113 (51–349)	164 (61–267)	0.92

AE, adverse event; NSAID, non-steroidal anti-inflammatory drug.

Fig. 1. Frequency (log_{10} scale) of emesis reported in dogs and cats in association with oral or injectable NSAIDs. Points represent frequencies for individual drugs and line indicates median.

Table 7
Spearman correlation coefficients for individual adverse events related to the number of years since granting of marketing authorisation.

AE	Spearman correlation coefficient (r_s)	*P*
Emesis	−0.793	0.005
Death	−0.691	0.002
Anorexia	−0.889	0.0005
Lethargy	−0.911	0.0005
Diarrhoea	−0.611	0.114
Hepatopathy	−0.675	0.069
Renal insufficiency	−0.916	0.002
Haemorrhagic diarrhoea	−0.611	0.114
Melaena	−0.431	0.218
Haematemesis	−0.339	0.359
Polydipsia	−0.943	0.017
Convulsions	−0.111	0.840

AE, adverse event.

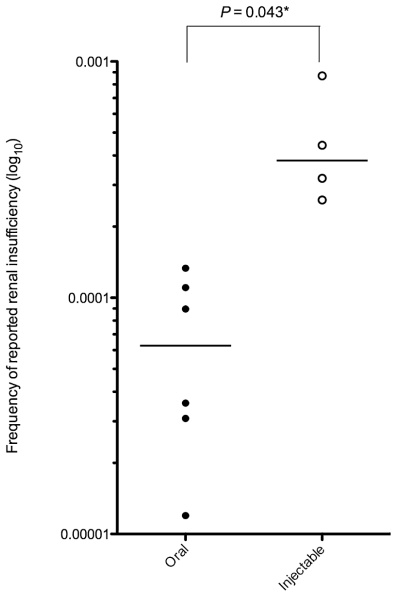

Fig. 2. Frequency (log_{10} scale) of renal insufficiency reported in dogs and cats in association with oral or injectable NSAIDs. Points represent frequencies for individual drugs and line indicates median.

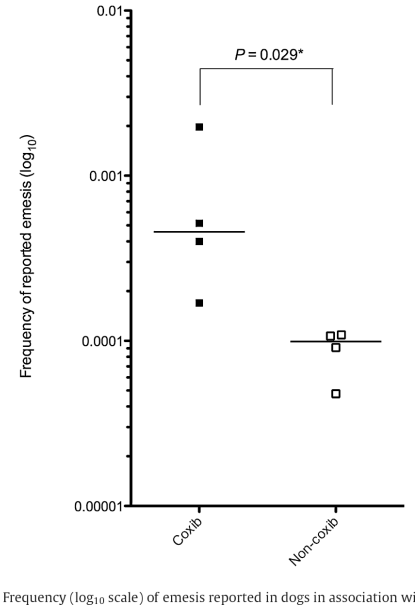

Fig. 4. Frequency (log_{10} scale) of emesis reported in dogs in association with coxib or non-coxib NSAIDs. Points represent frequencies for individual drugs and line indicates median.

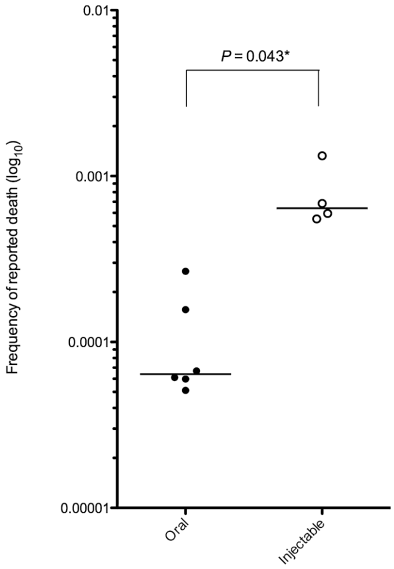

Fig. 3. Frequency (log_{10} scale) of death reported in dogs and cats in association with oral or injectable NSAIDs. Points represent frequencies for individual drugs and line indicates median.

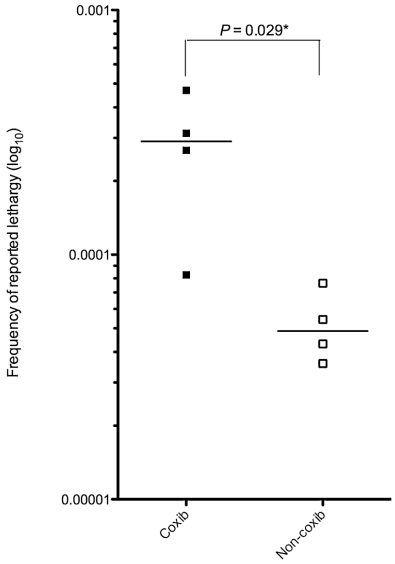

Fig. 5. Frequency (log_{10} scale) of lethargy reported in dogs in association with coxib or non-coxib NSAIDs. Points represent frequencies for individual drugs and line indicates median.

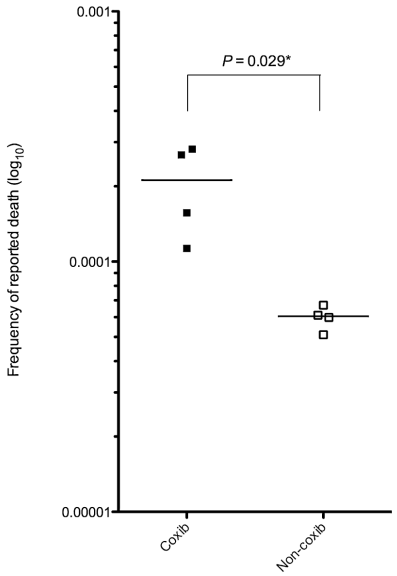

Fig. 6. Frequency (log_{10} scale) of death reported in dogs in association with coxib or non-coxib NSAIDs. Points represent frequencies for individual drugs and line indicates median.

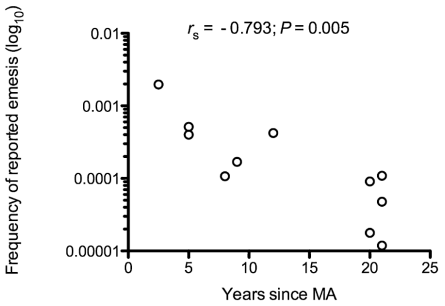

Fig. 7. Frequency (log_{10} scale) of emesis reported in dogs and cats plotted against time since marketing authorisation in years, demonstrating negative correlation.

Discussion

These data represent the most complete assessment possible of AE reports following NSAID administration in companion species in the UK. Pain management, which may include NSAID prescription, represents a significant contribution of the veterinary profession to animal welfare (Lascelles and Main, 2002). Knowledge of the frequency of reported NSAID AEs contributes to the ability of prescribers to balance benefits with risks of treatment, and informs discussions with animal owners. Until more active surveillance of AEs to NSAIDs is possible, the data presented are vital to aid decision-making around administration.

The frequency of reported NSAID AEs was low, and similar between molecules. Injectable formulations, compared with oral formulations, and coxibs compared with non-coxibs, were associated with a higher frequency of reported AEs. However, there are likely to be biases related to the circumstances in which the formulations were prescribed and AEs reported, which may well account for these differences.

Gastrointestinal disturbance, renal insufficiency, anorexia, lethargy and death were the most commonly reported AEs in cats and dogs; the data do not suggest either species to be at increased risk of any of these AEs. A systematic review of NSAID AEs in dogs by Monteiro-Steagall et al. (2013) reported that gastrointestinal AEs were most common, but these authors found renal insufficiency and death were not reported, suggesting those AEs were unlikely to occur within small to moderate studies. Owing to their seriousness, these AEs, if observed during clinical use, are likely to be reported and so appear relatively frequently in pharmacovigilance data. This supports the validity of assessing the frequency of reported AEs segregated by VeDDRA coding, rather than by assessing the total number of reports.

The frequency of emesis was less than one report per 500 doses of product sold. In clinical studies in dogs the incidence of vomiting has ranged from 1 in 25[5] to 1 in 4[6] (Reymond et al., 2012). Gastrointestinal disorders are well recognised (often self-limiting) NSAID-related AEs that are detailed in SPCs of all veterinary NSAIDs. Emesis may therefore be regarded as a 'normal' side effect of NSAIDs, thus the likelihood of reporting is low; the frequency reported here probably underestimates the likelihood of emesis associated with NSAIDs. Under-reporting of AEs within spontaneous reporting systems is well documented; in medical practice within the UK it has been estimated to be up to 98% (Fletcher, 1991). However, the degree of under-reporting decreases with increasing severity of AE, shorter length of time post MA, and the occurrence of an unlisted AE (Alvarez-Requejo et al., 1998).

Frequency of death reported was less than one per 500 injectable doses, and one per 2000 oral doses sold, however death ranked highly in the order of AEs for all of the NSAID molecules and formulations. Convulsions were reported with the majority of NSAID molecules, and have not previously been reported associated with licensed NSAIDs in dogs and cats. Seizures associated with ibuprofen and aspirin have been reported in the human medical literature (Hernández-Díaz and Rodríguez, 2000); additionally, the potential for certain NSAID molecules to potentiate epileptogenic γ-aminobutyric acid (GABA) antagonism of certain fluoroquinolone antibiotics has been reported (Kim et al., 2009). These data are insufficient to conclude that NSAIDs have the potential to increase convulsive activity in dogs and cats, but highlight the value of surveillance systems in detecting 'signal' data of unanticipated AEs coincident with product administration, which may then be more rigorously investigated (Williams, 2012).

Renal insufficiency was reported at low frequency in association with NSAID administration. Marino et al. (2014) reported that subclinical chronic kidney disease was identified in 50% of 86, randomly selected, client owned cats in the USA. It is possible that a significant population of cats harbour subclinical renal disease, and decompensation may occur coincident with an event which decreases glomerular filtration rate such as illness leading to dehydration (Greene et al., 2014). Whilst the frequency of reported renal insufficiency associated with NSAID administration may thus be artificially increased, it is advisable to ascertain hydration status prior to prescribing NSAIDs. The prevalence of clinical signs

[5] See: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm118041.pdf (accessed 27 February 2015).

[6] See: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm118026.pdf (accessed 13 February 2013).

of renal disease in dogs in the UK has been reported to be 0.37% (O'Neill et al., 2013), however there is currently no published information on the prevalence of subclinical renal disease in dogs.

Injectable formulations of NSAIDs were associated with increased reporting of death and renal insufficiency, compared with oral formulations, and are commonly administered perioperatively. The incidence of mortality related to general anaesthesia in healthy cats and dogs has been reported as 1 in 900 and 1 in 2000, respectively (Brodbelt, 2006). It is possible that a number of the reported deaths associated with injectable NSAIDs were also associated with anaesthesia and/or surgery, confounding the interpretation of these data. Risk of renal injury is increased by episodes of hypotension, such as may develop under general anaesthesia (Jones and Budsberg, 2000). Experimentally, the combination of hypotensive anaesthesia and meloxicam did not produce renal insufficiency in dogs studied for 7 days following anaesthesia (Boström et al., 2006). Therefore it is likely that individual patient or environmental factors determine the initiation and extent of renal injury. Balanced anaesthesia and appropriate intravenous fluid administration (to maintain adequate mean arterial blood pressure) are likely to be the most effective strategy to decrease risk of renal injury associated with perioperative NSAID use (Oliver et al., 1981).

Although coxibs were associated with higher reported frequencies of lethargy, emesis and death than non-coxibs, these data probably suffer from bias related to the duration of MA. Compared with non-coxibs, coxibs have a shorter duration of MA and a negative correlation was observed between the reported frequency of AEs and the duration of MA. The original description of increased reporting of drug related AEs during the early post-MA period, followed by decreased reporting with time, was provided by Weber (1984), and these trends continue to be identifiable (Wallenstein and Fife, 2001; Hartnell and Wilson, 2004; Chhabra et al., 2013). Our data support previous findings that, with increasing MA duration, reporting of AEs declines. Although data relating to NSAIDs with a longer MA would also be expected to be affected by the Weber effect, a longer duration post initial marketing would decrease the cumulative frequency of AEs, resulting in the observed differences between more recently licensed and more established drugs.

There was a higher frequency of reported AEs associated with mavacoxib. As one administration of mavacoxib produces similar efficacy to 30 separate doses of a daily-administered NSAID (Cox et al., 2011), the reported frequency of AEs associated with each administration of mavacoxib would be expected to be approximately 30× higher than a daily-administered product of similar MA duration, and these results support this assumption. The apparent increased frequency of AEs with mavacoxib is likely to be caused by presentation of data on a per dose basis.

There are caveats to the interpretation of our data. Due to commercial sensitivity, and data protection, only the frequency of reported VeDDRA terms as a proportion of the number of doses sold since authorisation is presented. The estimate of the number of doses sold was an assumption, but any resulting inaccuracy was applied equally, allowing meaningful comparison of the frequencies between NSAID molecules and formulations. It was not possible to evaluate an untreated group to describe the frequency of AEs in this population. Association between product administration and AE occurrence does not demonstrate causality so the degree to which reported AEs were attributable to NSAID administration is uncertain. However, the majority of frequently reported AEs in this study correlate with documented effects of NSAIDs (Monteiro-Steagall et al., 2013).

PSUR data were not available for short periods for a small number of NSAID formulations. It is likely that these data related to transition periods between brand names, and did not represent active sales periods (G. Davis, personal communication, 2014). The marked increase in overall reporting of AEs to the VMD is likely to introduce bias into our data, which will be most pronounced for more recently licensed molecules. This may further contribute to the negative correlation identified between the duration of MA and the frequency of reported AEs.

Current data do not identify patient risk factors, such as increasing age, which may predispose to AEs. There is a need to undertake a large-scale prospective cohort study, conducted within first-opinion veterinary practice, in order to generate robust data on the incidence of NSAID related AEs. However, passive surveillance data are valuable in comparing products that would be expected to be subject to similar reporting biases, and in directing future research efforts. Frequencies of AEs identified in this dataset provide information that can be used to generate hypotheses for prospective studies. The more complete reporting of these data is important because both veterinarians and owners collate the AE information for the VMD, and the information should be shared with those that contributed to it.

Conflict of interest statement

Elanco Animal Health funded the research post of James Hunt but played no role in the study design, in the collection, analysis and interpretation of data, or in the manuscript writing or submission for publication. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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2.4 Investigating Mechanisms of Pain in Canine Osteoarthritis

In addition to negatively affecting quality of life and potentially leading to early euthanasia of a significant number of pet dogs (Moore et al., 2001), naturally occurring osteoarthritis (OA) has been proposed as a model for the human disease (Vainio, 2012). Importantly the pain arising from joint pathology may become exaggerated by aberrant processing (central sensitisation, CS) within the central nervous system (Neogi, 2013). Whilst joint pain associated with OA commonly responds well to NSAIDs, cases which exhibit central sensitisation are likely to require classes of analgesics which interact with targets expressed in the central nervous system. However, currently available clinical metrology instruments designed to evaluate pain in dogs are unable to differentiate localised joint pain from central sensitisation. Characterisation of pain processing in canine osteoarthritis is therefore desirable in order to treat canine disease adequately, and additionally to increase the potential for the translation of canine research to human medicine. Quantitative sensory testing (QST) in conscious dogs has demonstrated group-level somatosensory sensitisation in dogs affected by naturally occurring osteoarthritis, compared with a group of unaffected dogs, suggestive of central sensitisation or generalised peripheral somatosensory hyperalgesia (Knazovicky et al., 2016). However, generating clinically useful ranges for expected responses in individual healthy dogs comparable to human data (Rolke et al., 2006) is likely to require significant effort, given that individual subject factors including bodyweight and condition, age, and sex have been shown to impact nociceptive thresholds (Harris et al., 2015; Knazovicky et al., 2016; Sanchis-Mora et al., 2017). Limitations of QST methods in dogs have been reported as poor feasibility in approximately 17% of dogs (Williams et al., 2014; Briley et al., 2014), effects due to the temperament and degree of

anxiety of the dog (Moore, S.A. et al. 2013), and the subjective interpretation of behavioural responses as an end point.

Nociceptive withdrawal reflexes (NWR) represent co-ordinated activation and inhibition of motor units as a consequence of afferent nociceptive impulses, with the evolutionary objective of avoiding potentially tissue damaging stimuli. The co-ordination of NWR is dependent on the site of noxious stimulation (Schouenborg et al., 1994). The reflexes occur at the level of the spinal cord, but are subject to descending inhibition and facilitation (Harris & Clarke, 2003). Recording of effector muscle electromyographic (EMG) responses of nociceptive withdrawal reflexes represents a quantitative method of recording the magnitude of motor response to a stimulus (Carstens & Ansley, 1993). Muscle depolarisation and contraction produces a positive and negative deflection of electrical potential recorded by paired intramuscular electrodes, relative to an earth electrode. This deflection may be recorded over a finite period of time. Assessment of the magnitude of an EMG response could therefore be based upon the maximum amplitude of change in potential, the duration of change in potential, or the product of these (integral; magnitude x time area under the curve). In the case of EMG recordings in response to NWR from sensitised subjects, changes could occur in both the magnitude and the duration of the deflection. Simply evaluating the mean of positive and negative components would reduce the calculated amplitude, therefore EMG signals are rectified (negative deviations converted to positive) (Kamen and Caldwell, 1996). EMG amplitudes may also be susceptible to artefact (Kamen and Caldwell, 1996), therefore calculation of the integral diminishes the

impact of high amplitude, low duration artefacts, whilst providing reliable estimate of myogenic activation over time.

Central sensitisation has been evaluated in humans using EMG based techniques (Banic et al., 2004) and, in experimental animal models of OA, NWR facilitation (indicating CS) has been demonstrated (Kelly et al., 2013). Previously published work has assessed NWR in trained conscious or sedated research dogs (Bergadano et al., 2006; Bergadano et al., 2009). In order to be clinically applicable, such methods would need to be tolerated by client owned dogs, which are likely to be less tractable. EMG responses to nociceptive stimuli could be obscured by movement artefact in conscious or sedated dogs. Obtaining EMG recordings of sufficient quality, and minimising aversive experiences associated with the application of noxious stimuli (which, in addition to animal welfare considerations, may further modulate nociceptive transmission (Fanselow, 1986; Rhudy & Meagher, 2000)) would therefore most probably necessitate anaesthesia in client owned dogs.

Diffuse noxious inhibitory controls (DNIC), the correlate of conditioned pain modulation (CPM) in humans (Yarnitsky, 2010), represent an descending endogenous inhibition of wide dynamic range neurons induced by a heterotopic noxious ('conditioning') stimulus (Le Bars et al., 1979; Harris, 2016). Descending noradrenergic neurons originating within the locus coereleus represent a key effector of these descending controls, whilst serotonergic neurons originating within the rostral ventromedial medulla produce pro- or anti-nociceptive effects at the dorsal horn, dependant on the 5-HT receptor subtypes activated (Bannister et al., 2017). CPM is assessed experimentally in man by measuring the magnitude of decrease in pain sensation, increase in pain threshold (Le Bars et al., 1991), or reduction

in nociceptive withdrawal reflex magnitude (Jurth et al., 2014) to a noxious ('test') stimulus, following application of the conditioning stimulus.

DNIC has been elicited in animals using a number of conditioning stimuli including mechanical, thermal, electrical, and chemical (Le Bars et al., 1979). In man a variety of stimuli have been applied in conscious volunteers to elicit CPM, including immersion of the hand in hot water (Granot et al., 2008), intramuscular hypertonic saline (Arendt-Nielsen et al., 2008), ischaemia (Fujii et al., 2006), and cutaneous application of capsaicin (de Tommaso et al., 2007). However the most commonly reported method of activating endogenous descending analgesia is immersion of the hand in iced water (the 'cold pressor test', CPT) (Baad-Hansen et al., 2005; Pud et al., 2009).

Considering the evidence of widespread central sensitisation to nociceptive stimuli in dogs affected by spontaneous hindlimb OA (Knazovicky et al., 2016); it must be recognised that secondary hyperalgesia may occur as the net outcome of either impaired descending inhibition or augmented facilitation of nociception (Millan, 2002; Bannister et al., 2015). To better evaluate contributory mechanisms to a global pain state, it would be desirable to be able to differentiate the functional status of descending inhibitory and facilitatory pathways. A reliable and reproducible protocol for the induction of DNIC in dogs had not been published prior to undertaking these investigations.

During a three year BBSRC funded research project, our aims were to develop a protocol for the evaluation of EMG in anaesthetised dogs, to determine whether EMG recording

demonstrated evidence of sensitisation of NWR in dogs affected by naturally occurring osteoarthritis, to develop an experimental protocol for the induction of DNIC in anaesthetised dogs, and determine whether this was altered in dogs with OA, and to assess whether these findings correlated with owner completed metrology instruments, clinical evaluation, and QST.

2.4.1 Paper 4. Hunt, J., Murrell, J., Knazovicky, D., Harris, J., Kelly, S., Knowles, T.G., Lascelles, B.D.X., 2016. Alfaxalone Anaesthesia Facilitates Electrophysiological Recordings of Nociceptive Withdrawal Reflexes in Dogs (Canis familiaris). PLoS ONE 11, e0158990.

The first step towards investigating nociceptive processing in dogs required us to develop and characterise a suitable general anaesthetic protocol which could be utilised in client owned dogs in the United Kingdom. Pilot work completed under the Animal (Scientific Procedures) Act 1986, amended 2013, and conducted at Huntingdon Life Sciences demonstrated that, at concentrations required to prevent arousal, both isoflurane inhalational anaesthesia and propofol intravenous anaesthesia were unsuitable agents as EMG responses to nociceptive stimulation were abolished. These findings stand in contrast to a study by Lervik et al. (2012), in which NWR evoked EMG responses were recorded during isoflurane anaesthesia. Total intravenous anaesthesia, using alfaxalone solubilised in cyclodextrin, had previously been employed in rats to facilitate EMG recordings (Kelly et al.,

2013). Our aim was to develop a suitable alfaxalone based anaesthetic protocol that was appropriate for administration to client owned dogs, and which would enable us to record EMG data whilst minimising noise due to movement artefact. We performed a prospective experimental study at North Carolina State University, using seven purpose bred male hounds who underwent NWR recording protocol under three different conditions: acepromazine sedation; alfaxalone sedation; and alfaxalone anaesthesia. Alfaxalone sedation (infusion range 0.044–0.08 mg kg^{-1} min^{-1}) was defined as a reduced level of arousal, sufficient to maintain lateral recumbency, but retaining pharyngeal and largyneal reflexes. Alfaxalone anaesthesia (infusion range 0.075–0.1 mg kg^{-1} min^{-1}) was defined as a loss of consciousness and suppression of pharyngeal/laryngeal reflexes sufficient to permit and maintain endotracheal intubation. The order of treatments was randomised, but the investigators were aware of the treatment group. The study was approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC).

The magnitude of the cranial tibial EMG responses to differing protocols of increasing mechanical (von Frey filament) and electrical (increasing current, repeated suprathreshold at 60 second intervals, and repeated suprathreshold at 1Hz (designed to stimulate C-fibre wind-up)) stimulation of the skin and dermal tissues of the pelvic limb fourth digit were compared between the three different states in order to determine the effects of alfaxalone at sedative or anaesthetic doses on the EMG response. A multi-level modelling approach was used to analyse the data, to account for the hierarchical nature of the experiments. The results of the study demonstrated that, compared with acepromazine sedation, both alfaxalone sedation and anaesthesia increased nociceptive withdrawal thresholds and reduced the magnitude of EMG response to electrical nociceptive stimulation, whilst

alfaxalone anaesthesia reduced the magnitude of EMG response to mechanical nociceptive stimulation. Importantly, stimulus-response and temporal summation characteristics were preserved under both alfaxalone conditions, indicating that these techniques had potential utility in interrogating spinal processing of the NWR in dogs.

Concurrent with these experiments, and approved under the same IACUC permit, we began to evaluate ice water immersion of the manus as a means of eliciting DNIC in dogs under acepromazine or alfaxalone sedation, or alfaxalone general anaesthesia. We hypothesised that the application of the cold water stimulus would elicit DNIC, resulting in a decreased magnitude of EMG response produced by a defined electrical nociceptive stimulus.

Following completion of the previously described NWR protocols, electrical stimulation was delivered at 5 minute intervals and EMG responses recorded. Following three baseline readings, a conditioning stimulus comprising immersion of the manus and carpus in iced water (0-4^{o}C) for a period of 20 minutes was applied to the distal left forelimb, which had been previously shaved and degreased with soap. Interdigital skin temperature measurements were recorded every 2 minutes using a thermocouple. Time constraints (maximum testing duration specified within IACUC conditions) meant that data collection was performed for seven dogs during acepromazine sedation, four dogs during alfaxalone sedation, and five dogs during alfaxalone anaesthesia. Consistent with our earlier results, EMG magnitude was significantly greater in acepromazine sedated dogs compared with alfaxalone treated dogs. The mean interdigital skin web temperature across the 3 states immediately prior to immersion in ice water was 31.9^{o}C (95% CI 27.5 – 36.6^{o}C). Skin temperatures reached a minimum of 4.9^{o}C (95% CI 1.3 – 8.4^{o}C), 13.6^{o}C (95% CI 9.8 – 17.3^{o}C), and 9.4^{o}C (95% CI 5.9 – 12.9^{o}C) in acepromazine, alfaxalone sedated and alfaxalone anaesthetised states respectively, after 10 minutes ice water immersion, after which time

they began to rise despite being maintained in the water bath for a further 10 minutes. Skin temperature measurements remained significantly higher in the alfaxalone sedated state compared to the acepromazine state during immersion (p=0.018); there was no significant difference between alfaxalone groups (p=0.25). However, the application of the conditioning stimulus had no effect on EMG magnitude (figures 1 and 2), suggesting that the stimulus did not elicit DNIC. Potential explanations for this finding are that the noxious cold stimulus was of insufficient intensity to recruit DNIC in dogs; that the magnitude of the test stimulus was too great compared to the conditioning stimulus, causing the nociceptive withdrawal response to the test stimulus to overcome DNIC effects, or that the time period during which DNIC was effective did not align with the timings of our test stimuli.

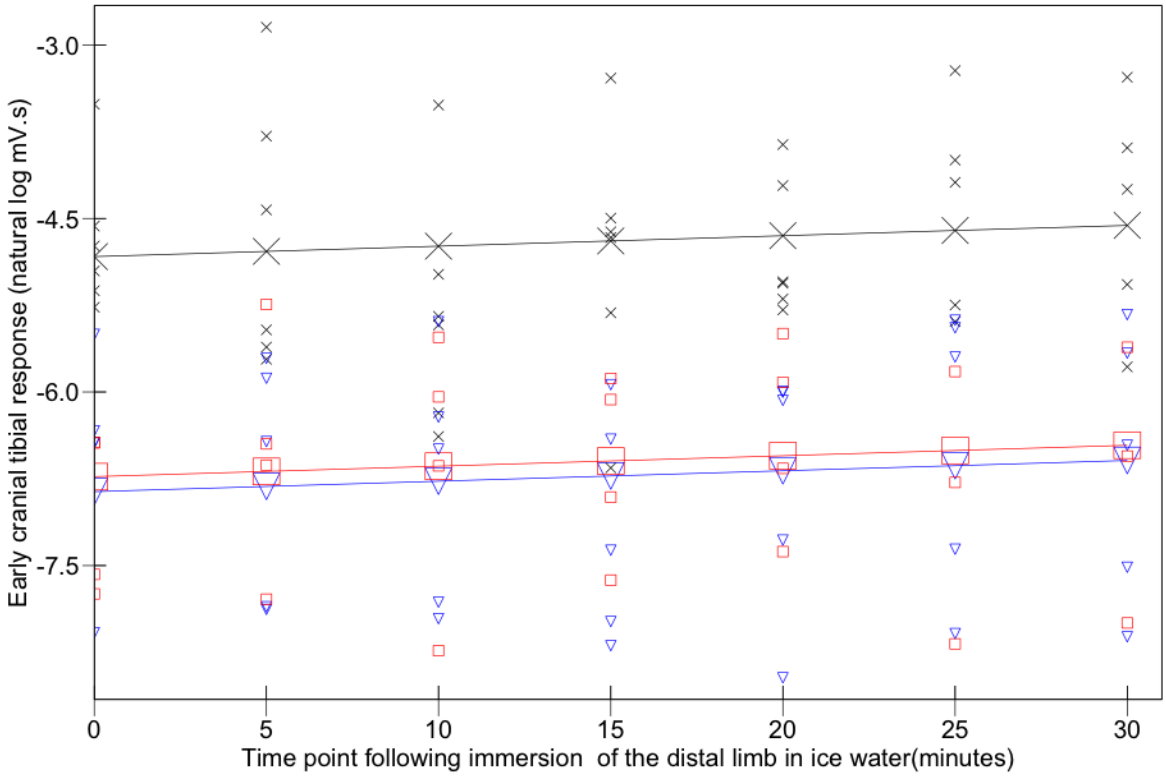

Figure 1 Early (0-100ms) cranial tibial EMG responses during cold water immersion of the manus. Time point 0 represents the baseline (mean of responses at T-15, -10, -5, and 0). Acepromazine (n=7) sedation is represented by X, alfaxalone anaesthesia (n=5) by ▽and alfaxalone sedation (n=4) by □. Recorded values are represented by small symbols, mean results are represented by larger symbols connected by lines. Variation with time was not significant. (An effective DNIC response would be illustrated by reduced magnitude of early cranial tibial response during the period of ice water immersion).

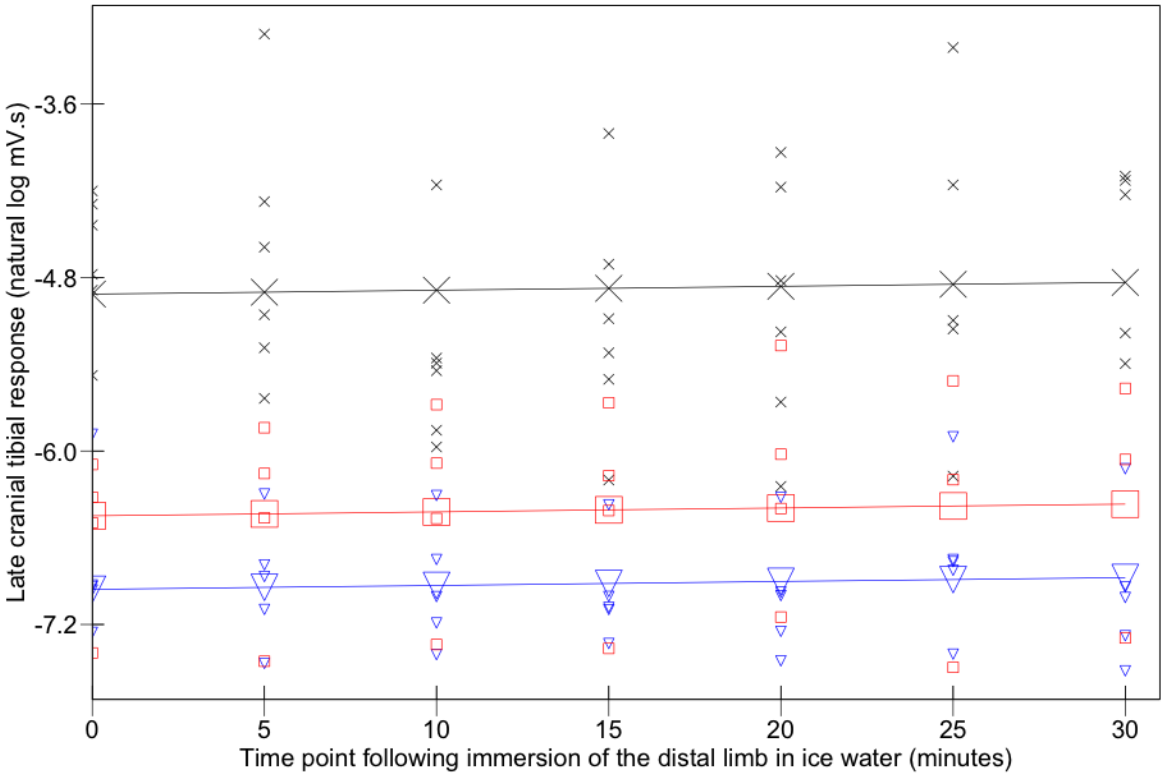

Figure 2 Late (100-500ms) cranial tibial responses. Time point 0 represents the baseline (mean of responses at T-15, -10, -5, and 0). Acepromazine (n=7) sedation is represented by X, alfaxalone anaesthesia (n=5) by ▽ and alfaxalone sedation (n=4) by □. Recorded values are represented by small symbols, mean results are represented by larger symbols connected by lines. Variation with time was not significant. (An effective DNIC response would be illustrated by reduced magnitude of late cranial tibial response during the period of ice water immersion).

DNIC is elicited only by higher intensity (moderate and severe) noxious conditioning stimuli (Nirl et al., 2012). Conscious, acepromazine sedated dogs responded to the conditioning stimulus by attempting to withdraw their limb from the stimulus, implying that the stimulus was perceived as aversive. However in the absence of a verbal report, the intensity of the conditioning stimulus was challenging to assess. Increasing the intensity (Nirl et al., 2012) or spatial distribution of the noxious conditioning stimulus (Bouhassira et al. 1995; Marchand & Arsenault 2002) can increase the recruitment of DNIC, though there is a maximum at which no further increase in descending inhibition is possible (Nirl et al. 2012). Application of the cold stimulus in this study to the level of the manus reflects the paradigm utilised in

man (Wolf & Hardy 1941), but it is possible that increasing the spatial distribution of the cold stimulus (i.e. stimulation over a greater area) would have been more likely to recruit DNIC.

Investigations of conditioned pain modulation in man have employed a test stimulus which produces a subjective pain experience of magnitude 60 out of 100 using a numerical rating scale (Nirl et al. 2012), whilst effective conditioning stimuli have produced subjective pain experiences of magnitude 31.57 ± (SD) 9.56 out of 100 (moderate intensity) and 58.1 ± (SD) 11.43 (severe intensity) (Nirl et al. 2012). It is possible that the electrical test stimulus used in this study was of a magnitude greater than the equivalent of 60 out of 100 in man, and generated a more robust nociceptive withdrawal response, suppression of which may have therefore been more difficult to induce. However, the stimulus chosen (5 x 5mA, 100Hz) produced a response approximating 50-60 % of maximum in our previous findings, therefore would have been expected to be of an appropriate magnitude to be applied as a test stimulus.

The cold pressor test in man is reported to provoke 90-95% of the total reduction in skin temperature within the first minute (Wolf & Hardy 1941). Mourot et al. (2009) reported skin temperatures of 19 male subjects undergoing CPT, which were measured as 14.3 ± (SD) 4.6^{o}C and 13.4 ± (SD) 5.1^{o}C following 2 and 3 minutes immersion respectively. We observed a much slower decline in skin temperatures, compared to studies of CPT in man, however the skin temperatures recorded in the present study reached similar temperatures to those reported by Mourot et al. (2009) at their nadir. Therefore, if skin temperature alone is the governing factor in the initiation of noxious cold related DNIC we would have expected to

see inhibition of the EMG responses at the 10 minute interval. Wolf & Hardy (1941) reported reduced pain in subjects that experienced slower cooling of the water bath, therefore it may be that the slow rate of change of skin temperature reflected a stimulus of insufficient magnitude to induce DNIC. Anatomical features identified in the footpads of dogs, including dermal arteriovenous anastomoses, a dermal venous plexus, and a thick layer of subcutaneous adipose tissue (Ninomiya et al. 2011) have been proposed to be adaptations to a cold environment. It is possible that, despite skin temperatures falling during immersion of the distal limb in ice water, these adaptations prevented tissue temperatures at the depth of cold nociceptors from falling to a degree necessary to represent a moderate or severe nociceptive stimulus.

Both acepromazine (Alvaides et al., 2008) and alfaxalone (Rodríguez et al. 2012) exhibit vasodilator activity, which may have countered cold induced vasoconstriction, however administration of vasodilators by Wolf & Hardy (1941) did not alter the cold induced pain sensations in their subjects.

Endogenous analgesia following the cold pressor test is reported to persist for at least 3-8 minutes (Serrao et al. 2004), therefore, had we been able to induce DNIC, we would expect that test stimuli delivered at 5 minute intervals would be appropriate to demonstrate evidence of suppression of the NWR. We did not deliver test stimuli more frequently, due to the concern that more frequent stimulations would result in sensitisation of the NWR. However, our work confirming the stability of responses to stimuli delivered at 60 second intervals suggest that this frequency does not induce sensitisation over a period of 10

minutes, and therefore more frequent test stimuli could be employed in future DNIC studies, which would increase the sensitivity of the technique to short-lived effects.

Acepromazine has central and peripheral antagonist activity at dopamine, α - adrenergic, 5-hydroxytryptamine and histamine receptors, in addition to decreasing synaptic uptake of adenosine (Booth 1988), but is not considered to have analgesic or antinociceptive properties (Bergadano et al. 2009). Alfaxalone is reported to be devoid of analgesic activity (Winter et al. 2003), and its effects are mediated by potentiation of gamma-amino butyric acid (GABA) (Warne et al. 2015). DNIC is reportedly unaffected by GABA agonists (Kunz et al. 2006); acepromazine sedation and alfaxalone sedation or anaesthesia would therefore be expected to provide suitable conditions for investigation of DNIC. However, there are no published studies describing the effects of different anaesthetics on CPM efficiency and testing characteristics, therefore, the possibility remains that sedation and anaesthesia could have modulated the expected responses. Reassuringly DNIC has been demonstrated in other species utilising a variety of anaesthetic agents to produce unconsciousness. In halothane anaesthetised rats DNIC has been successfully induced using noxious mechanical force at a number of body sites, noxious heat and electrical stimulation of the tail and intraperitoneal bradykinin (Le Bars et al. 1979), whilst in pentobarbitone anaesthetised rabbits cutaneous application of mustard oil to the snout has been used to induce DNIC (Harris 2016).

In order to safeguard the welfare of animals exposed to a conditioning stimulus, the stimulus should be rapidly terminated, non-tissue damaging and rapidly effective,

permitting a short testing time. For these reasons, mechanical or ischaemic induced noxious conditioning stimuli were considered the most rewarding modalities to investigate further. These investigations into cold-water elicited DNIC in dogs were presented as a poster abstract at the International Association for the Study of Pain World Congress on Pain.

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RESEARCH ARTICLE

Alfaxalone Anaesthesia Facilitates Electrophysiological Recordings of Nociceptive Withdrawal Reflexes in Dogs (*Canis familiaris*)

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Abstract

Naturally occurring canine osteoarthritis represents a welfare issue for affected dogs (*Canis familiaris*), but is also considered very similar to human osteoarthritis and has therefore been proposed as a model of disease in humans. Central sensitisation is recognized in human osteoarthritis sufferers but identification in dogs is challenging. Electromyographic measurement of responses to nociceptive stimulation represents a potential means of investigating alterations in central nociceptive processing, and has been evaluated in conscious experimental dogs, but is likely to be aversive. Development of a suitable anaesthetic protocol in experimental dogs, which facilitated electrophysiological nociceptive withdrawal reflex assessment, may increase the acceptability of using the technique in owned dogs with naturally occurring osteoarthritis. Seven purpose bred male hound dogs underwent electromyographic recording sessions in each of three states: acepromazine sedation, alfaxalone sedation, and alfaxalone anaesthesia. Electromyographic responses to escalating mechanical and electrical, and repeated electrical, stimuli were recorded. Subsequently the integral of both early and late rectified responses was calculated. Natural logarithms of the integral values were analysed within and between the three states using multi level modeling. Alfaxalone increased nociceptive thresholds and decreased the magnitude of recorded responses, but characteristics of increasing responses with increasing stimulus magnitude were preserved. Behavioural signs of anxiety were noted in two out of seven dogs during recordings in the acepromazine sedated state. There were few significant differences in response magnitude or nociceptive threshold between the two alfaxalone states. Following acepromazine premedication, induction of anaesthesia with 1–2 mg kg^{-1} alfaxalone, followed by a continuous rate infusion in the range 0.075–0.1 mg kg^{-1} min^{-1}

no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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produced suitable conditions to enable assessment of spinal nociceptive processing in dogs, without subjecting them to potentially aversive experiences. This methodology may be appropriate for obtaining electrophysiological nociceptive withdrawal reflex data in client-owned dogs with naturally occurring osteoarthritis.

Introduction

Osteoarthritis (OA) has been estimated to affect 20% of adult dogs (*Canis familiaris*), and the resulting pain presents a significant welfare challenge in the management of older animals [1]. Naturally occurring canine OA is most commonly secondary to joint dysplasia or abnormal loading forces and progresses over a time course. Canine OA shares developmental, pathological and clinical features with human OA [2]. In addition, client owned dogs are likely to be exposed to similar environmental conditions [3], and potentially lifestyle factors [4], as human OA sufferers. Spontaneously occurring canine OA has therefore been proposed as a model for further investigation of, and trial of therapeutics for the human condition, with the potential to impact both animal and human welfare [5]. If this model is to be optimized, all relevant outcome measures and measures of the pain state need to be developed and validated, including measures of central sensitisation.

The development of enhanced nociceptive transmission within the central nervous system (central sensitisation, CS) is a feature of OA in a subset of human sufferers [6], and may predict a poor response to present standard of care analgesics [7]. In humans CS can be characterised by the application of a variety of sensory modalities [7] and the presence of CS is indicated by secondary mechanical dynamic and punctate hyperalgesia. Central sensitisation has also been suspected in clinical canine OA patients [8]; however there is presently no standard method of assessing CS in dogs, therefore the fidelity of the spontaneous canine OA model to human OA cannot be assured. Furthermore, there is the potential for varying and unknown degrees of CS to confound the results of therapeutic trials in dogs. Behavioural responses have been assessed in order to evaluate potential alterations in nociceptive thresholds to heat, pressure, and cold (quantitative sensory testing, QST) in dogs experiencing OA and have indicated heat hypoalgesia [9], mechanical hyperalgesia [10,11] and cold hyperalgesia [11]. Limitations of QST methods in dogs have been reported as lack of feasibility in some dogs [9,12], effects due to the temperament and degree of anxiety of the dog [13], and the subjective interpretation of behavioural responses as an end point.

Nociceptive withdrawal reflexes (NWR) were first described as gross unified limb flexion movements in response to suprathreshold nociceptive stimulation by Sherrington [14]. Subsequent work however has characterised responses as co-ordinated activation and inhibition of both flexor and extensor muscles in a limb, with the resultant co-ordination dependent on the site of noxious stimulation [15,16]. NWR in individual hindlimb muscles can therefore be recorded using electromyography (EMG) in order to obtain a quantitative measurement of the motor response to a peripherally applied stimulus [17] and to study the organization of responses in individual muscles to stimulation at a particular site. Central sensitisation appears to enhance the protective movement provided by individual muscle NWRs [18]. Central sensitisation in chronic pain states in humans has been evaluated using NWR methodology [19], and CS has been demonstrated via NWR facilitation in experimental animal models of OA [20]. Measurement of NWR may be a more objective methodology than QST to gain insight into central nervous system plasticity in dogs and therefore may have utility in client-owned dogs with chronic pain conditions, including OA. Nociceptive withdrawal reflex methodology

in client-owned dogs would also facilitate clinical trial evaluation of putative analgesics that target modulation of CS.

Previously published work has assessed NWR in conscious, trained research dogs, but client owned dogs are likely to be less tractable and artefacts associated with movement are likely to obscure EMG responses to nociceptive stimuli. Collection of EMG recordings of sufficient quality, whilst minimising the aversive experience associated with the stimulation (which, aside from animal welfare considerations, may, in itself, modulate nociceptive transmission [21,22]) may necessitate anaesthesia in such dogs. Recordings of EMGs elicited by both high frequency (200Hz) train-of-5 electrical stimuli [23], and lower frequency (2, 5 and 20 Hz) repeated stimuli [24] have been described in pain free conscious and acepromazine sedated [25] dogs; and proposed as an objective means of investigating central nervous system plasticity [25] as well as the effects of analgesic drugs [26]. Previous work has shown that a low dose ($0.01 mg\ kg^{-1}$) of acepromazine administered intravenously to experimental dogs produced mild tranquilisation for approximately 30 minutes and did not alter the characteristics of the recorded EMG [25]. However acepromazine sedation alone may not be considered to produce an appropriate degree of anxiolysis prior to the application of repeated nociceptive stimuli to client owned dogs. Sedation with dexmedetomidine may produce more effective anxiolysis but would be expected to affect NWR [27]. General anaesthesia would obviate any potential stress and awareness of nociception associated with NWR protocols, thus refining the procedure. It would also likely increase owner acceptability of the procedure, particularly if electrical stimuli are used. Preliminary pilot work investigating the ability to record EMGs in dogs anaesthetised with isoflurane, indicated that isoflurane concentrations required to prevent arousal abolished EMG responses in a significant number of tests. Alfaxalone anaesthesia has been employed in laboratory animal species to facilitate EMG recordings [20]. The specific aim of this current study was to develop a suitable alfaxalone based anaesthetic protocol for use in client owned dogs, which would enable us to record EMG data whilst minimizing movement artefact.

The primary outcome measures of this study were the magnitudes of the recorded EMGs; these were compared in order to determine the effects of sedation or anaesthesia produced by alfaxalone infusion on the recorded EMG, in comparison with results collected in acepromazine sedated dogs.

Materials and Methods

Seven purpose bred male hound dogs (weight range 25.5–29.2 kg) were used for the NWR/EMG recordings. The dogs were housed individually within enclosures of dimensions approximately 1.5 x 4.6 metres, and 2.7 metres high, with enrichment consisting of toys that are changed out and rotated on a regular basis and standard bedding. The dogs' exercise and socialisation program consisted of two 30 minute walks each day, outside, and two 20 minute play and socialization periods each day. The dogs were maintained on a standard complete dog food and food was withheld for eight hours prior to administration of sedative drugs. Ethical approval was obtained prior to beginning the study from the North Carolina State University Institutional Animal Care and Use Committee (IACUC; permit number 13-010-B). The experimental protocol was performed with the dogs restrained or anaesthetised in right lateral recumbency on a synthetic bedding, beneath which was a circulating warm water blanket and padded table top. Following completion of EMG recordings, carprofen ($4 mg\ kg^{-1}$) (Rimadyl, Zoetis, New Jersey, USA) was administered subcutaneously to treat any ongoing pain associated with the stimulation and recording protocols. Following completion of these experiments the dogs continued to be kept at the North Carolina State University Animal Care Facility to participate in further research projects.

Placement of EMG recording electrodes

The left pes was rested on a sandbag to support the limb perpendicular to the table top. Stainless steel needle recording electrodes (disposable subdermal needle electrode 12 x 0.40 mm, Natus Neurology Inc. Middleton, WI, USA) were placed transcutaneously, in pairs, into the belly of the cranial tibial (CT), biceps femoris (BF), and sartorius (SA) muscles of the left pelvic limb. A ground electrode for each pair of recording electrodes was inserted subcutaneously, dorsal to the dorsal spinous process of L6. The locations of the electrodes are shown in Fig 1. The distance between each of the electrodes in the pair was as follows: cranial tibial 18mm; biceps femoris 21mm; sartorius 21mm. Recording electrodes were connected to an analogue to digital converter (Powerlab 4/35, AD instruments, Oxford, UK) via separate differential amplifiers (DAM50, World Precision Instruments, Herts, UK) which applied a low pass filter of 10 Hz, high pass filter of 1 KHz, and gain of 1000 to the signal. The resulting digital signal was captured and recorded using Labchart 8 software (AD instruments, Oxford, UK) run on a

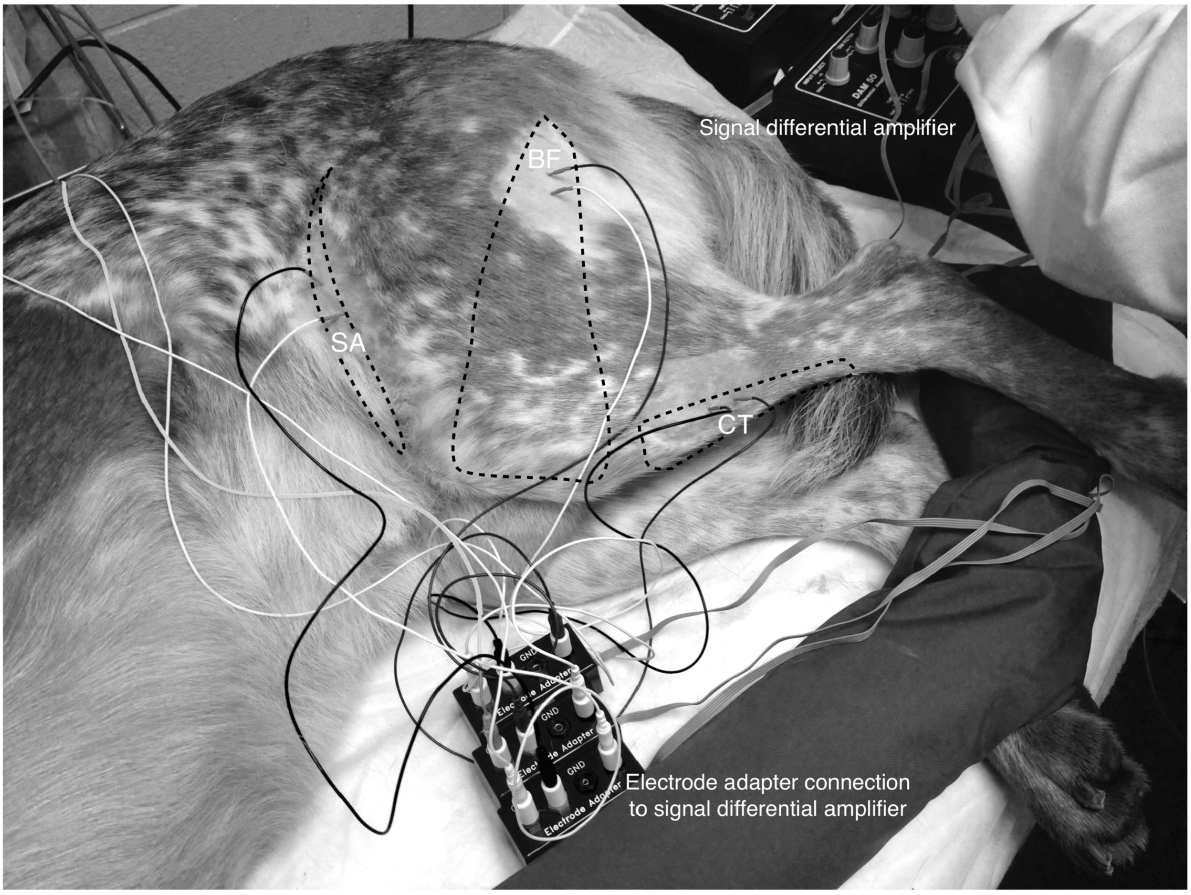

Fig 1. Demonstrating the position of recording electrodes in cranial tibial (CT), biceps femoris (BF) and sartorius (SA) muscles, together with ground electrode placement subcutaneously over the dorsal lumbar region.

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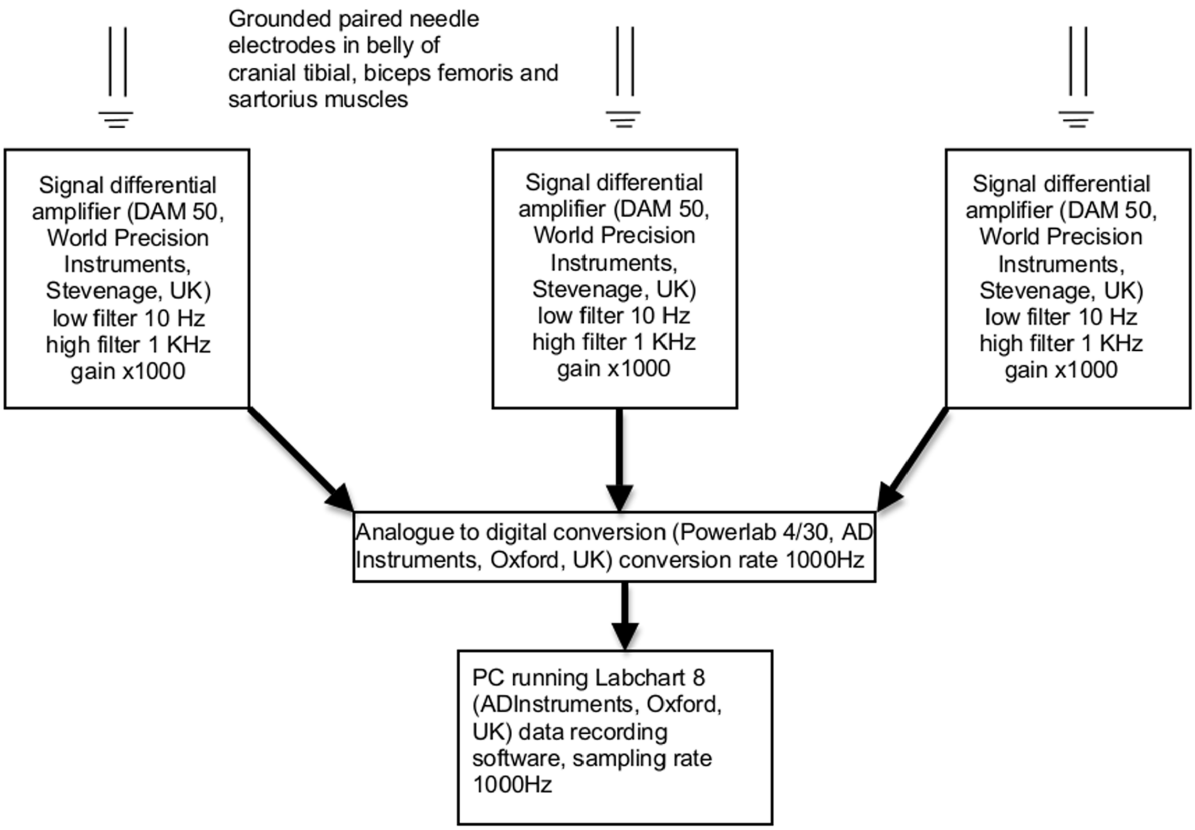

Fig 2. Schematic, illustrating recording connections.

doi:10.1371/journal.pone.0158990.g002

Toshiba Tecra R850 (Toshiba Europe, 41460 Neuss, Germany) laptop, utilising Windows 7 operating system (Microsoft Corp., USA). Fig 2 is a schematic illustrating the recording connections used to measure NWRs.

Sedation and anaesthesia

All dogs underwent the EMG recording procedure described below in each of three states: acepromazine sedation, alfaxalone sedation or alfaxalone anaesthesia.

In the acepromazine sedated state, dogs were administered acepromazine (Acepromazine Maleate Injection, Boehringer Ingelheim Vetmedica, Missouri, USA) 0.03 mg kg^{-1} injected intramuscularly into the lumbar longissimus dorsi, 30 minutes prior to being instrumented for EMG recording. The alfaxalone sedation state was produced by administration of an intravenous bolus of 1mg kg^{-1} alfaxalone (Alfaxan, Jurox, Missouri, USA), 30 minutes following acepromazine premedication as above. Sedation was maintained by intravenous infusion of alfaxalone (range 0.044–0.08 mg kg^{-1} min^{-1}); rate of infusion was adjusted to produce a state in which dogs assumed lateral recumbency, were non-responsive to quiet auditory stimuli, and exhibited decreased muscle tone but maintained laryngeal reflexes. Dogs in this state were

provided with supplemental oxygen at a rate of 1–2 litres min^{-1} via a close fitting facemask. The alfaxalone anaesthesia state was achieved by administration of an intravenous bolus of 1–2 mg kg^{-1} alfaxalone, 30 minutes following acepromazine premedication, as above, injected slowly until adequate conditions for orotracheal intubation were achieved. Anaesthesia was maintained by intravenous infusion of alfaxalone; rate of infusion was adjusted to produce a state in which dogs exhibited depressed muscle tone and laryngeal reflexes, sufficient to maintain orotracheal intubation with a cuffed endotracheal tube through which oxygen was delivered via a circle rebreathing system. The rate of alfaxalone infusion (range 0.075–0.1 mg kg^{-1} min^{-1}) was adjusted to preserve a slow palpebral reflex. Following completion of EMG recordings, alfaxalone infusions were discontinued and dogs were monitored during recovery to the point that they were able to walk unaided, at which point they were transported in a wheeled crate back to their kennel. Examination the following day was performed to ensure no evidence of tissue damage or ongoing pain. Due to technical issues, dog 1 did not undergo testing in the alfaxalone sedation state and dog 5 did not undergo testing in the alfaxalone anaesthesia state.

Stimulation protocol

Determination of the most sensitive area. Rat tooth forceps were used to deliver a firm pinch, sequentially to 21 points on the skin (Fig 3) on the plantar aspect of the left pes, whilst recordings of EMG activity were performed, to determine the most sensitive area (i.e. that which generated the largest magnitude EMG response) to a mechanical nociceptive stimulus. This sensitive area was then used to apply further mechanical and electrical stimuli. The results of this part of the experiment indicated that the most sensitive area was the area just proximal to the pad of the fourth digit (location 14 on Fig 3), and this area was used in subsequent stimulation protocols.

Mechanical Stimulus Response Curve. In order to construct an EMG stimulus response curve mechanical stimulation was delivered by the application of von Frey filaments (North Coast Medical, Inc., California, USA) in ascending weight order; 4, 6, 8, 10, 15, 26, 60, 100, 180 and 300g. Each filament was applied perpendicularly to the skin of the previously defined most sensitive area of the pes with a force just sufficient to cause the filament to bend. This position was maintained for 3 seconds, before removing the filament from contact with the skin. The complete series of von Frey filaments was applied in the same ascending order on a total of 3 occasions, with 5-minute intervals between each train of von Frey stimulation.

Response to Electrical Stimulation. Following completion of mechanical stimulation, a pair of stimulating needle electrodes (disposable subdermal needle electrode 12 x 0.40 mm, Natus Neurology Inc. Middleton, WI, USA) was placed into the plantar dermal tissues of the distal phalanx of the fourth digit, immediately proximal to the proximal edge of the digital pad, of the left pelvic limb. Electrical stimuli were delivered using a constant current stimulator from an isolated 100 V source (Stimulus isolator FE180, AD instruments, Oxford, UK).

(i) Electrical Stimulus Response Curve

One stimulus event comprised five 1 ms stimuli (Train-of-5, To5), which were delivered at a frequency of 100 Hz. In order to construct an EMG stimulus response curve, To5 events were triggered at 60-second intervals using currents of 0.1 (baseline), 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mA. The complete series of stimulating currents were applied in the same ascending order on a total of 3 occasions, with 5-minute intervals between each occasion.

(ii) Response Stability Testing

For assessment of stability of the EMG response, 10 To5 events were delivered at 60- second intervals with a fixed current of 10 mA.

(iii) Temporal Summation

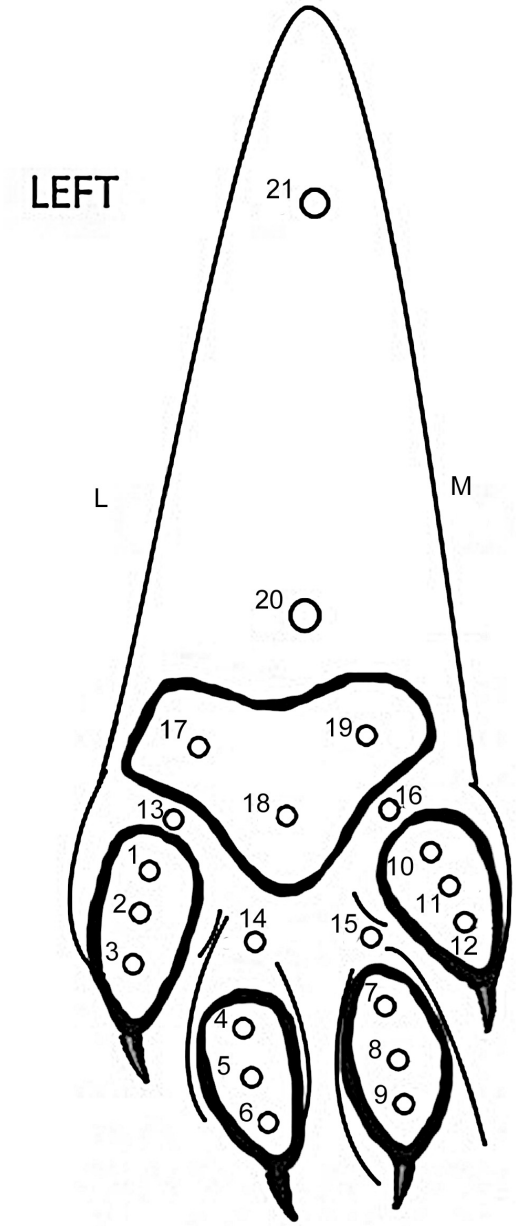

Fig 3. Diagram of left pes illustrating 21 points assessed for sensitivity by pinching with rat tooth forceps. L, lateral; M, medial.

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Subsequently, the effect of temporal summation was induced by a stimulus train, comprising eight single 10 mA stimuli delivered at a frequency of 1 Hz. This stimulation protocol was delivered on three occasions at five-minute intervals.

EMG analysis

The mechanical and electrical stimulus intensity which elicited a greater than baseline amplitude response of CT, was noted and defined as nociceptive threshold. Post recording, 10Hz digital filtering was applied to the EMG traces, to further decrease movement artefact. EMG recordings were assessed visually to identify the time intervals which represented an early (indicative of an A- fibre mediated [23]) and late (consistent with a C- fibre mediated) response. The early period was defined as 0–100 ms following stimulation, while the late period was defined as 100–500 ms following stimulation. The integral of the rectified EMG response was extracted for each stimulus. Natural log(ln) [integral] values were subsequently analysed using the statistics package MLwiN [28] which allowed the repeated measures structure of the data to be properly accommodated within the analyses. The statistical model was based on a structure consisting of an individual measurement within repetition, within each anaesthetic state, within dog and then the effect of anaesthetic state and magnitude of stimulation (electrical or mechanical) was tested within a general linear model. To test for a non-linear relationship a series of polynomial terms for the stimulation were specified. Terms were retained within the models if they were significant at $\alpha \leq 0.05$ and were tested against a change in the log likelihood using a Chi square distribution. The resulting significant models were presented as graphs of the ln response plotted against the level of stimulation in order to depict the mean response to the stimulation.

The effect sizes and *P* values of the predictor variables included in the final models were tabulated, allowing reconstruction of the figures from the predictive equations.

Duration of testing procedure, and median nociceptive thresholds, were compared between the three states using Krukal-Wallis test, and Dunn's multiple comparison test post-hoc.

Results

The median (range) duration of each recording session for each state was 200 (165–270) minutes, and there were no differences in recording duration between the three states ($P = 0.12$).

During acepromazine sedation EMG activity was invariably associated with gross movement with flexion of the hock, stifle and hip. During alfaxalone sedation and anaesthesia EMG activity was associated with small contractions of the cranial tibial muscle (visible as 'twitches'). EMG responses from the biceps femoris and sartorius muscles were infrequently recorded in all except the acepromazine sedated dogs. However it was possible to reliably and consistently record EMG responses from the cranial tibial muscle in each dog during each state of sedation or anaesthesia and so comparisons of data recorded were limited to the cranial tibial muscle.

Tolerability of the protocol

Two dogs exhibited signs of anxiety (lip licking, ears drawn back, attempting to escape) during the stimulation protocol whilst sedated with acepromazine. Electrode displacement requiring electrode repositioning was encountered during electrical stimulation in these 2 dogs during the acepromazine sedated state, due to instances of gross movement, but in none of the dogs during alfaxalone sedation or anaesthesia. EMG recordings were feasible in the two anxious dogs following reassurance by handlers.

Mechanical nociceptive threshold

Mechanical thresholds for individual dogs in each state, together with group medians, are shown in Table 1. Both alfaxalone treated groups exhibited significantly higher median mechanical thresholds ($P = 0.01$) compared to acepromazine sedated dogs, but thresholds were not different between alfaxalone groups.

Mechanical stimulation (von Frey) stimulus response curve

The effect sizes and *P* values of the predictor variables included in the final models are presented in S1 Table.

Recorded responses increased in magnitude with increasing stimulus weight (S1 Table, Figs 4 and 5). Alfaxalone anaesthesia decreased the magnitudes of the EMG responses recorded (Fig 5), compared with those recorded in both acepromazine and alfaxalone sedated dogs, and there was a significant interaction between stimulus weight and state ($P = <0.001$, S1 Table). The repetition of the stimulus response curve was not identified as a significant source of response variability in the final model, indicating that neither sensitisation nor habituation to the stimulus occurred.

Electrical nociceptive threshold

Electrical nociceptive thresholds for individual dogs in each state, and group medians, are shown in Table 1. Alfaxalone sedated dogs exhibited significantly higher median electrical thresholds ($P = 0.01$) compared to acepromazine sedated dogs, but thresholds were not different between alfaxalone groups, nor between acepromazine sedated and alfaxalone anaesthetised dogs. The characteristic response to electrical stimulation is illustrated in S1 Fig.

Table 1. Mechanical and electrical threshold recordings from the three states. *$P = 0.01$ compared to acepromazine threshold.

State	Dog number	Mechanical threshold g	Median mechanical threshold g	Electrical threshold mA	Median electrical threshold mA
Acepromazine sedation	1	15	15	2.5	2
	2	60		2	
	3	8		1	
	4	26		2	
	5	100		6	
	6	15		1.5	
	7	8		2	
Alfaxalone sedation	2	300	240*	7	5.5*
	3	60		4	
	4	180		3	
	5	300		4.5	
	6	300		8	
	7	180		6.5	
Alfaxalone anaesthesia	1	300	300*	3	3.5
	2	300		10	
	3	300		7	
	4	180		2	
	6	60		4	
	7	300		2	

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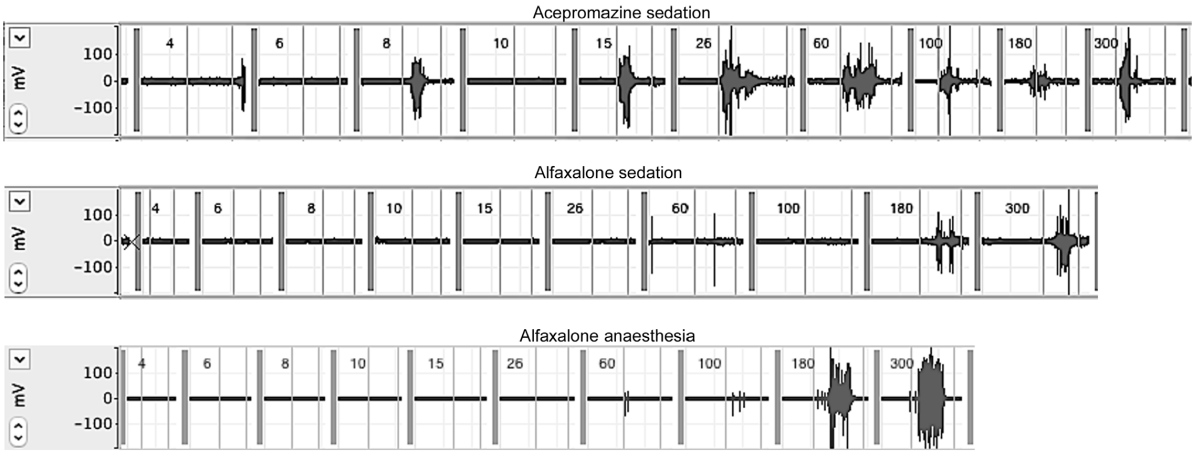

Fig 4. EMG recordings from cranial tibial muscle of dog 3 in response to application of increasing weights of von Frey filaments. Upper trace following acepromazine sedation, middle trace following alfaxalone sedation and lower trace following alfaxalone anaesthesia. Weights of von Frey filaments are shown on each trace in grams, thin grey vertical lines represent the period during which stimulation was applied at each weight.

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Electrical stimulus response curves

Increasing magnitudes of responses to increasing stimulating current were observed in both early and late responses in all three states (S1 Table, Fig 6A and 6B).

There was a significant interaction between current and state ($P = <0.001$, S1 Table); whilst the rate of increase in EMG response with current was initially higher (greater increase in response per unit increase in stimulus intensity) in acepromazine sedated dogs compared to alfaxalone treated dogs, the magnitude of EMG responses began to plateau at currents greater than 6mA in acepromazine sedated dogs, but continued to increase up to the maximal (10mA) stimulation in both alfaxalone treated groups. Repetition and individual dog were not significant causes of variability in response.

Electrical evoked response stability and comparison of EMG magnitude between states

The early and late EMG responses were repeatable (there was no significant difference in EMG magnitude between subsequent stimulus occasions) in acepromazine and alfaxalone sedated dogs (Fig 7A and 7B). However there was significant variation of the magnitude of early (but not late) EMG response with occasion in alfaxalone anaesthetised dogs ($P = 0.005$, S1 Table).

Compared to acepromazine sedated dogs, alfaxalone sedation and anaesthesia significantly decreased the magnitude of early and late EMG (P = 0.007, S1 Table).

Temporal Summation

The magnitude of the early EMG response (Fig 8A) to the temporal summation stimulation protocol decreased with increasing occasion number in the acepromazine sedated dogs, yet remained constant with increasing occasion in both alfaxalone states. The magnitude of the late EMG response (Fig 8B) increased in all groups at a comparable rate (slopes are parallel). Compared to the acepromazine sedated group, the magnitude of the early and late EMG

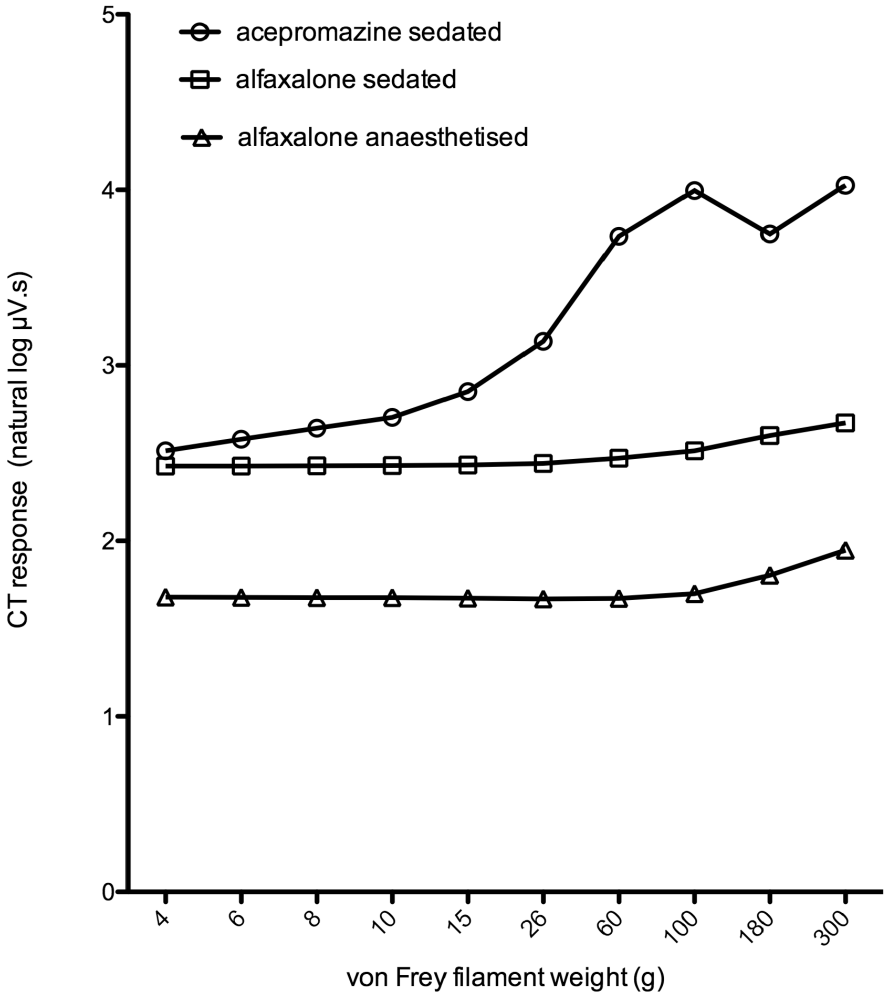

Fig 5. Responses to 3 second application of increasing weights of von Frey filament to the plantar skin of digit 4 in Fig 3.

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responses recorded in the alfaxalone sedated and anaesthetised groups was significantly decreased ($P = <0.001$, S1 Table).

There was an obvious difference in behavioural response of the dogs in the different states to temporal summation, with the acepromazine sedated dogs maintaining their stimulated limb in flexion with repeated delivery of the stimulus.

Discussion

We have demonstrated that it is possible to record robust and repeatable EMGs in response to mechanical and electrical nociceptive stimuli in dogs administered alfaxalone. This represents the first investigation of the effect of anaesthetic state on recorded EMG responses in dogs and

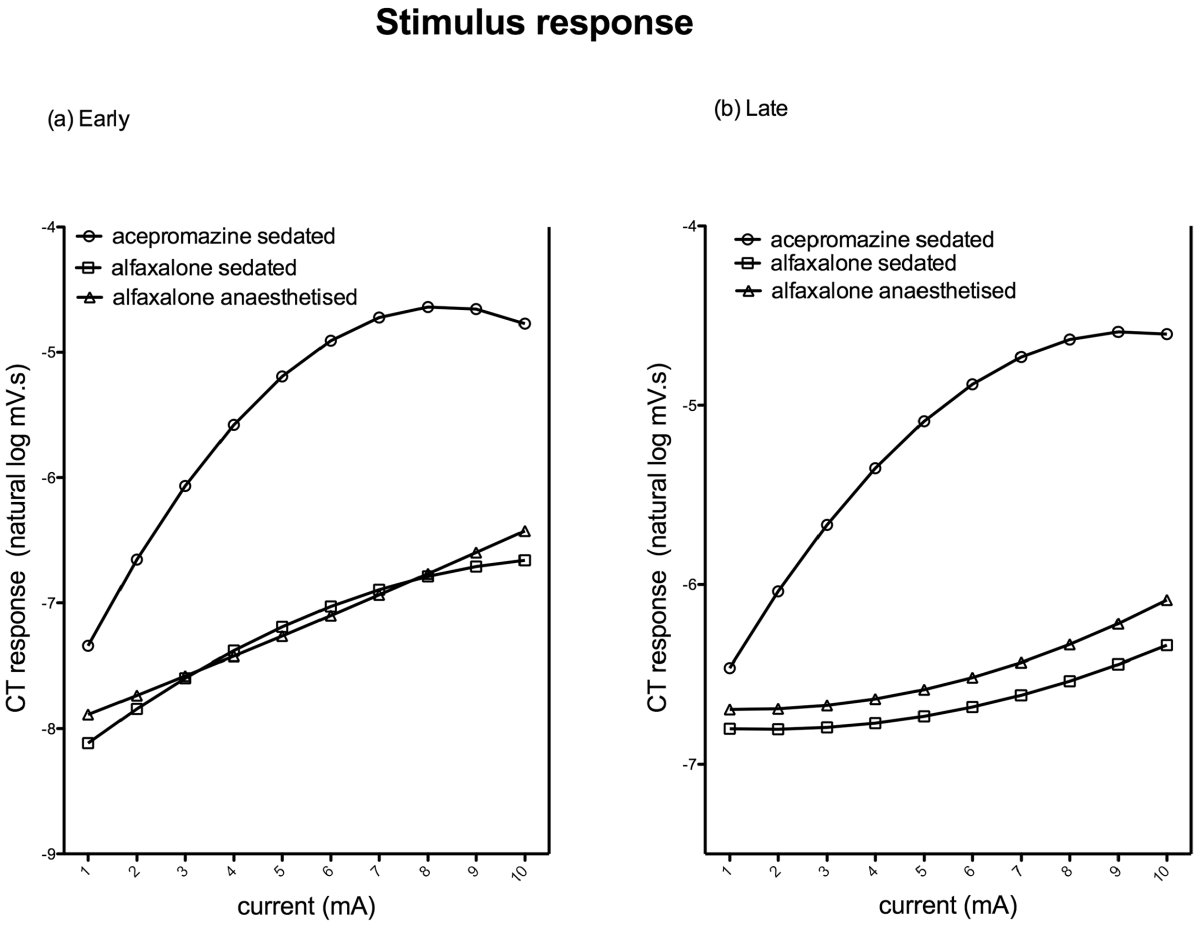

Fig 6. Mean EMG responses to electrical stimulus response; early 0-100ms (a) and late 100-500ms (b). Responses of the acepromazine sedated group are represented by ○, alfaxalone sedated by □, and alfaxalone anaesthetised by △.

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provides a methodological platform for future studies examining CS in dogs with naturally occurring OA pain. Alfaxalone anaesthesia decreased the magnitude of EMGs recorded in response to mechanical stimuli, and both alfaxalone treatments decreased the magnitude of EMGs in response to electrical stimuli, compared to EMGs recorded in dogs sedated with acepromazine only. However, responses remained readily identifiable, and continued to demonstrate expected stimulus response and temporal summation characteristics during both alfaxalone states. Furthermore there were no significant differences between the magnitudes of response to electrical stimulation between the two alfaxalone treatment groups. Thus, if NWR/EMG responses are to be recorded in client owned animals, general anaesthesia may be successfully employed in order to facilitate recording, and negate the aversive experiences associated with the stimuli. However, the stability data suggest that the magnitude of EMG response varies with anaesthetic state, therefore our results indicate that maintaining a stable plane of

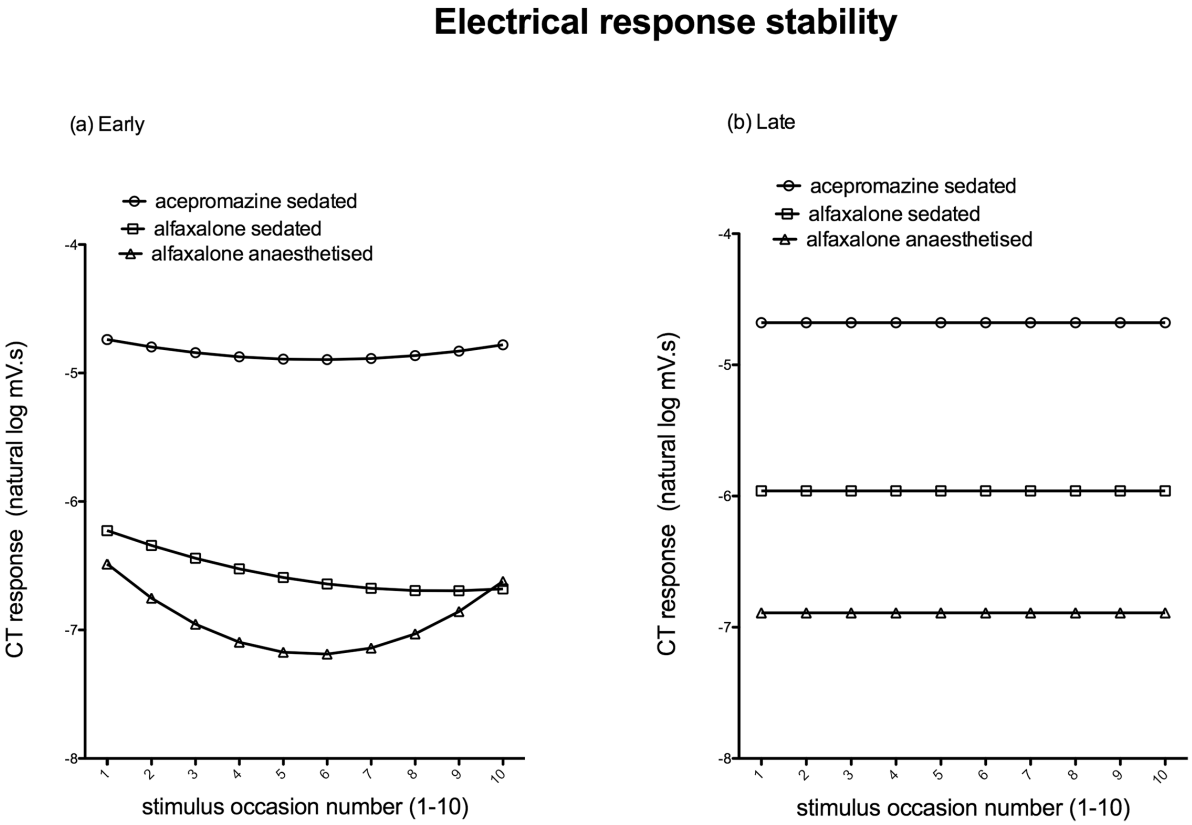

Fig 7. Mean EMG responses to electrical stability; early 0-100ms (a) and late 100-500ms (b). Responses of the acepromazine sedated group are represented by ○, alfaxalone sedated by □, and alfaxalone anaesthetised by △.

doi:10.1371/journal.pone.0158990.g007

anaesthesia is critical for NWR/EMG recording, and is most likely to be achieved by continuous rate, or appropriately modelled target controlled, infusion. Generation and recording of EMG responses to electrical nociceptive stimuli in acepromazine sedated, pain free dogs replicates previous findings [25], supporting the contention that such a technique may be able to be transferred to the clinical setting to describe nociceptive processing in individual pet dogs. However behavioural signs, which could indicate anxiety, and electrode displacement resulting from subject movement, were noted more frequently in this state, which may limit the acceptability and utility of the procedure in client owned dogs. The recording protocol required the dogs to remain in lateral recumbency for 3 hours, which again may be more difficult to achieve without anaesthesia in client owned dogs, particularly those suffering from painful conditions. Whilst dog owners routinely consent to potentially aversive diagnostic testing in conscious dogs (e.g. venipuncture, fine needle aspiration of masses) these are expected to be brief experiences. A more prolonged procedure involving administration of repeated nociceptive stimuli may be expected to result in increased stress, compared to a momentary single aversive stimulus. Stress may inhibit [21] or potentiate [29] nociception. These psychological states represent potential confounding factors if NWR/EMG recording is attempted in non-anaesthetised dogs.

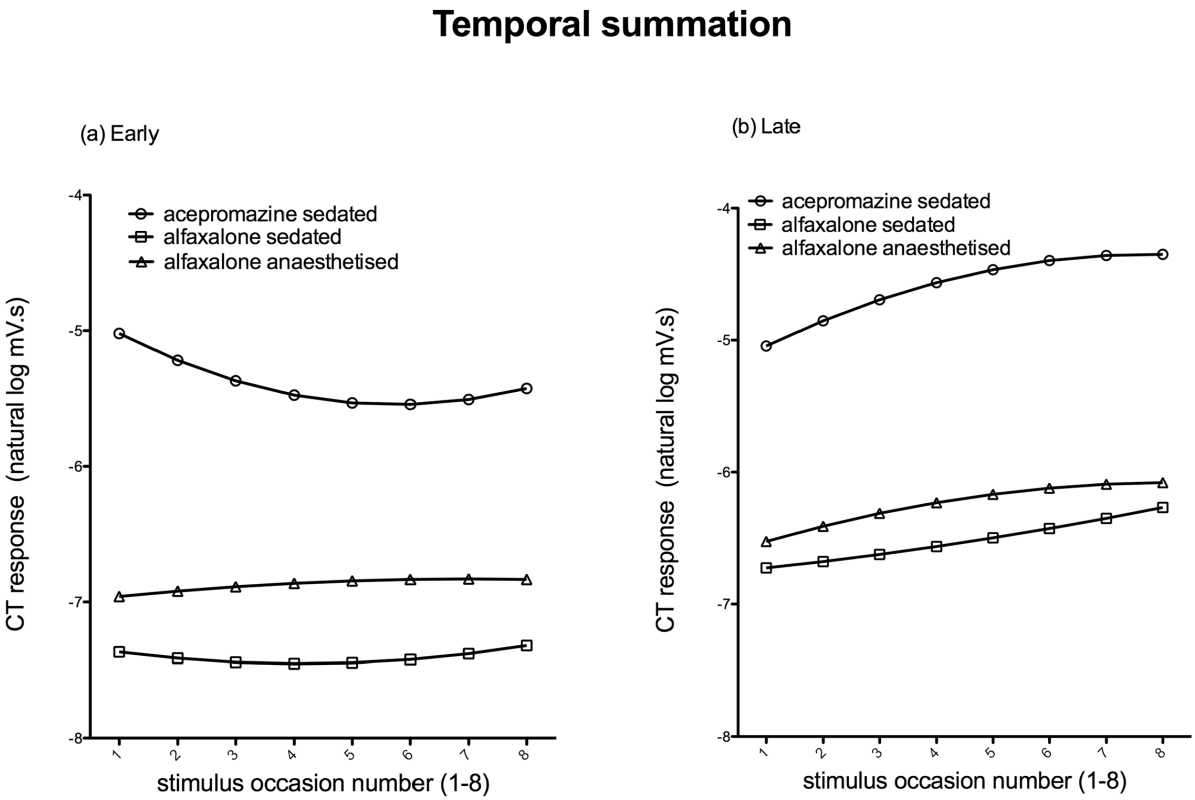

Fig 8. Mean EMG responses to temporal summation; early 0-100ms (a) and late 100-500ms (b). Responses of the acepromazine sedated group are represented by ○, alfaxalone sedated by □, and alfaxalone anaesthetised by △.

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There are a paucity of data available regarding the degrees of stress and welfare compromise involved in veterinary diagnostic procedures, however a semi-quantitative approach to assigning values to represent the welfare of dogs with respect to differing treatment options has been described [30]. A similar approach to assessing welfare impacts of differing diagnostic approaches could be employed, and in the absence of contra-indications, anaesthesia may represent a higher welfare method of achieving NWR evaluations.

Recording of EMG responses to nociceptive stimuli has previously been reported in dogs anaesthetised with isoflurane [27]; however our preliminary pilot work, using the methodology described above, indicated that isoflurane concentrations required to prevent arousal abolished EMG responses in a significant number of tests. Other investigators [31] reported that isoflurane anaesthesia depressed heat evoked withdrawal responses in rats, as measured by a force meter. In humans and ponies, NWR thresholds are significantly increased by isoflurane [32,33]. Isoflurane produces immobility at inspired concentrations of 0.8–1.2 minimum alveolar concentration, via preferential depression of ventral horn spinal neurones [34]. This property may limit its utility as an anaesthetic agent during procedures which require measurement of motor responses. Alfaxalone has previously been successfully employed as an anaesthetic agent during EMG recordings in rats [20,35] and, on this basis, was selected for this study in

dogs. However the effects of this agent on NWR and EMG recordings have not been previously studied in dogs, and therefore both alfaxalone sedation and alfaxalone anaesthesia were studied.

Different modalities of nociceptive stimuli may have differing utility in the measurement of NWR/EMG recordings. Application of punctate mechanical stimuli to the area of the pes exhibiting highest sensitivity was performed in order to evaluate the responses to a physiologically relevant noxious stimulus which generates action potentials via activation of A- and C-fibre mechanically sensitive afferents. Both alfaxalone treatments significantly increased the threshold force required to elicit a response. In addition, the magnitude of response was decreased by alfaxalone anaesthesia, compared to acepromazine or alfaxalone sedation. To our knowledge this is the first study to determine EMG responses to punctate mechanical stimuli in dogs. Given that central sensitisation results in secondary hyperalgesia to mechanical, but not heat stimuli [36], assessment of EMG responses to mechanical stimulation may be used as a specific means of assessing and quantifying central sensitisation [20]. In order to evaluate mechanical sensitivity in anaesthetised dogs, stimuli of greater magnitude may be necessary. Application of electrical stimuli represents a quantifiable and widely employed (e.g. [25,37,38]), but non-physiological, means of eliciting NWRs. Electrical stimulation obviates the transduction required by physiological stimuli, and depolarises both low and high threshold nociceptive fibres simultaneously [39], in addition to non-nociceptive afferents [40,41]. Such widespread activation of different fibres may itself result in modulation of nociception at the spinal cord [40]. In acepromazine and alfaxalone sedated states, responses were repeatable, demonstrating that this protocol represents a stable condition, in which the effects of altering stimulus parameters can be assessed over the period of the experiment. Unexpectedly, the magnitude of the early response in the alfaxalone anaesthetised dogs varied with occasion (repeat of the To5). This is a concern, as such an effect may confound determination of NWRs. A 10m A stimulus was chosen to assess electrical stability on the basis that this would represent a suprathreshold stimulus. The response stability data for the alfaxalone anaesthetised state was derived from only 5 of the 7 dogs and the threshold current during alfaxalone anaesthesia in dog 2 was determined to be 10 mA. Re-examining the EMG recorded from dog 2 it is evident that a number of stimuli during the response stability testing had produced no response (i.e. 10 mA represented a subthreshold stimulus). Repeating the analysis, following exclusion of the stability data from dog 2 during the alfaxalone anaesthetised state, demonstrated that the responses of the remaining alfaxalone anaesthetised dogs were repeatable in this state, and suggests that the protocol should be equally valid in alfaxalone anaesthetised dogs.

Alfaxalone increased the threshold to electrical stimuli, although this was only statistically significant in the alfaxalone sedated state. Alfaxalone also decreased the magnitude of resulting EMGs. However the characteristics of increasing responses associated with increasing intensity of stimuli, allowing the construction of stimulus-response and temporal summation curves, were maintained. Decreased NWR threshold (i.e. a left shift in the stimulus response curve) has been reported in human populations experiencing augmented pain as a result of central sensitisation [19]; the ability to generate stimulus-response curves in pain-free alfaxalone infused dogs offers potential as a method of investigating central sensitisation in dogs. Further work to define reference EMG magnitude values to predetermined increasing electrical stimuli in normal, non-painful dogs would be beneficial. In all states, increasing late responses (consistent with C-fibre activation) were observed in response to temporal summation, suggesting this protocol would be suitable for investigating wind-up in alfaxalone infused dogs. The decreasing early EMG magnitude with increasing stimulus number in the acepromazine dogs is likely to reflect the behavioural response—continued maximal flexion of the limb—thus limiting the potential for further muscle contraction. Augmentation of NWR in response to

temporal summation is regarded as a cardinal feature of central sensitisation, and has been reported in a number of clinical pain conditions in man [42,43].

Modular organisation of nociceptive withdrawal reflexes [16] produces greatest motor activity in specific muscles, activation of which would remove the stimulated area from the noxious stimulus. The most robust recordings in response to stimulation at point 14 were therefore made from the cranial tibial muscle, which would be the prime mover in flexing the tibiotarsal joint, removing the foot from the stimulus. It was not possible to record consistent EMG signals from the biceps femoris and sartorius muscles which are situated more distant to the site of stimulation. Higher intensity stimulation (e.g. temporal summation) did result in flexion of the hip and generation of EMG recordings from the sartorius (hip flexor) during acepromazine sedation, however responses were not observed in this muscle during alfaxalone infusions; comparisons were not, therefore, performed.

In conclusion, it was possible to record robust and reproducible EMG responses to electrical stimulation in both the alfaxalone states, and the results of the stimulus response and temporal summation stimulations were not different between the two states. Performing recordings in the alfaxalone anaesthesia state does not appear to negatively impact the data collected, and this state permits tracheal intubation, which is desirable in anaesthetised dogs. Following acepromazine premedication, induction of anaesthesia with 1–2 mg kg^{-1} alfaxalone, followed by a continuous rate infusion in the range 0.075–0.1 mg kg^{-1} min^{-1} has the potential to enable assessment of spinal nociceptive processing, by applying mechanical and electrical stimulus response, and electrical temporal summation protocols, in client owned dogs without subjecting them to potentially aversive experiences. Altered central nervous system nociceptive processing has been identified in a number of chronic pain conditions [44], and a means of investigating the nociceptive system may assist clinicians in targeting therapeutic interventions in individual animals.

Supporting Information

S1 Fig. Illustration of typical response evoked by train-of-five electrical stimulation and time domains during which measurements were performed.
(TIF)

S1 Table. Effect sizes and *P* values of the predictor variables (and interactions between variables) included in the final models.
(DOCX)

Author Contributions

Conceived and designed the experiments: JM J. Harris SK TGK BDXL. Performed the experiments: BDXL J. Hunt JM DK. Analyzed the data: J. Hunt TGK JM J. Harris BDXL. Contributed reagents/materials/analysis tools: SK J. Harris TGK BDXL J. Hunt JM. Wrote the paper: J. Hunt JM DK J. Harris SK TGK BDXL.

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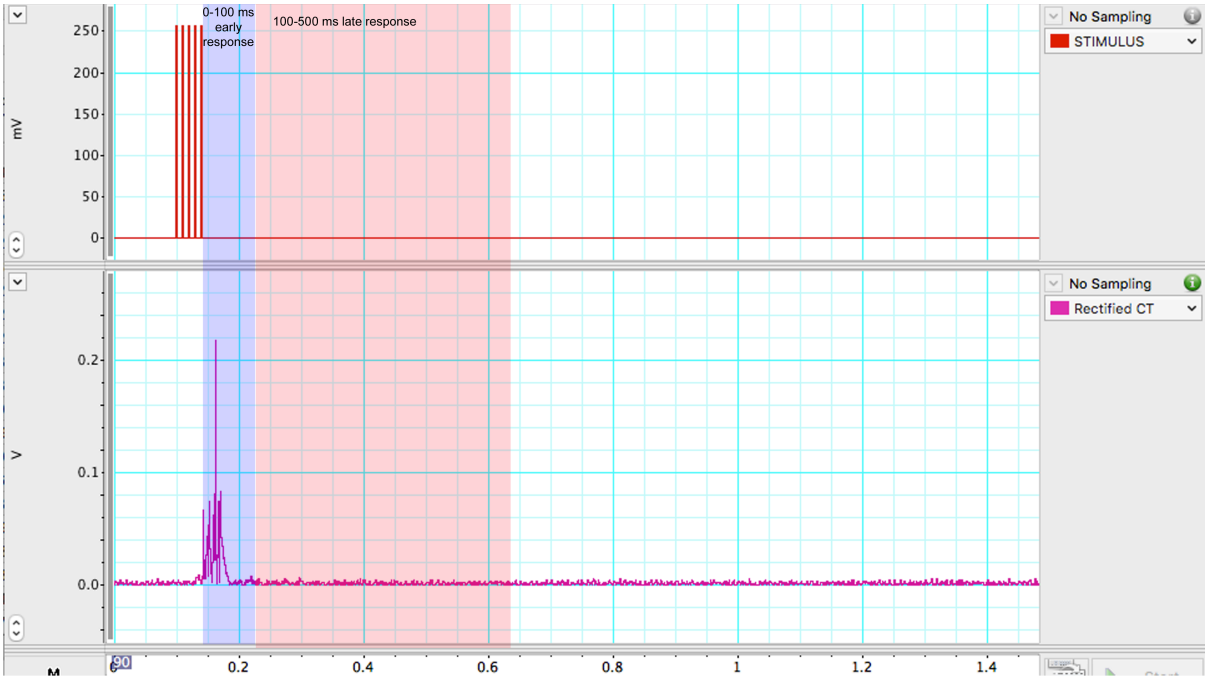

S1 Fig. Illustration of typical response evoked by train-of-five electrical stimulation and time domains during which measurements were performed.

Predictor variable	Mechanical			Electrical stimulus (early response)			Electrical stimulus (late response)			Electrical stability (early response)			Electrical stability (late response)			Temporal summation (early response)			Temporal summation (late response)		
	Ln(μV.S)	S.E.	P	Ln(mV.S)	S.E.	P	Ln(mV.S)	S.E.	P	Ln(mV.S)	S.E.	P	Ln(mV.S)	S.E.	P	Ln(mV.S)	S.E.	P	Ln(mV.S)	S.E.	P
Constant, recorded during ACP sedation (x=0)	2.3773069	0.2941749	-	-8.132	0.285	-	-6.945	0.207	-	-4.667	0.334	-	-4.678	0.346	-	-4.775	0.313	-	-5.264	0.287	-
Adjustment to constant by alfaxalone sedation	0.0461343	0.3698821	0.901	-0.283	0.314	0.367	0.161	0.260	0.536	-1.427	0.489	0.004*	-1.281	0.474	0.007*	-2.528	0.441	<0.001*	-1.504	0.442	<0.001*
Adjustment to constant by alfaxalone anaesthesia	-0.6934438	0.3928979	0.078	0.095	0.314	0.762	0.264	0.260	0.310	-1.491	0.512	0.004*	-2.211	0.502	<0.001*	-2.232	0.417	<0.001*	-1.393	0.420	<0.001*
Mechanical stimulus weight	0.0349592	0.0035281	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mechanical stimulus weight²	-0.0002324	0.0000330	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mechanical stimulus weight³	0.0000004	0.0000001	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone sedation x Stimulus weight	-0.0343866	0.0051932	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone anaesthesia x Stimulus weight	-0.0358420	0.0054657	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone sedation x Stimulus weight²	0.0002368	0.0000486	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone anaesthesia x Stimulus weight²	0.0002451	0.0000511	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone sedation x Stimulus weight³	-0.0000005	0.0000001	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone anaesthesia x Stimulus weight³	-0.0000005	0.0000001	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stimulus occasion	-	-	-	-	-	-	-	-	-	-0.078	0.064	0.223	-	-	-	-0.268	0.062	<0.001*	0.235	0.056	<0.001*
Stimulus occasion²	-	-	-	-	-	-	-	-	-	0.007	0.006	0.243	-	-	-	0.023	0.007	0.001*	-0.015	0.006	0.012*
Alfaxalone sedation x Occasion	-	-	-	-	-	-	-	-	-	-0.062	0.096	0.518	-	-	-	0.195	0.093	0.036*	-0.196	0.085	0.021*
Alfaxalone anaesthesia x Occasion	-	-	-	-	-	-	-	-	-	-0.281	0.099	0.005*	-	-	-	0.319	0.089	<0.001*	-0.095	0.081	0.241

Alfaxalone sedation x Occasion[a]	-	-	-	-	-	-	-	-	-	0.001	0.008	0.901	-	-	-	-0.015	0.010	0.134	0.018	0.009	0.046*
Alfaxalone anaesthesia x Occasion[a]	-	-	-	-	-	-	-	-	-	0.025	0.009	0.005*	-	-	-	-0.027	0.010	<0.001*	0.007	0.009	0.437
Stimulus current	-	-	-	0.840	0.065	<0.001*	0.508	0.042	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-
Stimulus current[c]	-	-	-	-0.050	0.006	<0.001*	-0.027	0.004	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone sedation x Current	-	-	-	-0.525	0.095	<0.001*	-0.532	0.062	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone anaesthesia x Current	-	-	-	-0.691	0.095	<0.001*	-0.529	0.062	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone sedation x Current[c]	-	-	-	0.036	0.008	<0.001*	0.034	0.005	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-
Alfaxalone anaesthesia x Current[c]	-	-	-	0.052	0.008	<0.001*	0.035	0.005	<0.001*	-	-	-	-	-	-	-	-	-	-	-	-

Table S1 Effect sizes and *P* values of the predictor variables (and interactions between variables) included in the final models

2.4.2 Paper 5. Hunt, J.R., Goff, M., Jenkins, H., Harris, J., Knowles, T.G., Lascelles, B.D.X., Enomoto, M., Mendl, M., Whay, H.R., Murrell, J.C., 2018. Electrophysiological characterisation of central sensitisation in canine spontaneous osteoarthritis. Pain 159, 2318–2330.

The development of EMG recording techniques suitable for use in anaesthetised dogs enabled the overarching project to proceed, with the aim of identifying electrophysiological changes in response to nociceptive stimuli in dogs affected by osteoarthritis, compared to an unaffected cohort. A prospective, non-blinded study was performed under the Animal (Scientific Procedures) Act 1986 (as amended, 2013) and the experimental protocol was approved by the University of Bristol Animal Welfare and Ethical Review Body.

Client owned dogs with suspected pelvic limb osteoarthritis were recruited via advertising within the University of Bristol, surrounding veterinary clinics, and via a dedicated Facebook study page.

Initially, we sought to recruit two groups of patients; those affected by OA (n = 100, anticipating that up to approximately 30% of these dogs may exhibit CS), and an age, breed, and sex matched control group (n = 35). However, during the recruitment phase it became apparent that a significant number of patients were currently being treated with NSAIDs. It was decided to recruit these animals without altering their daily NSAID treatment, but during data analysis these animals were identified as a separate group (OANSAID). We considered that this decision was justified given that pain and disability were still present in these individuals, despite analgesic treatment.

Interested dog owners were invited to an assessment visit where written and verbal descriptions of the projects aims and methods were provided. Owners who were willing to enroll their dog on the project were asked to sign a consent form but advised that they could withdraw at any time. I performed a clinical and musculoskeletal examination of all enrolled dogs, to rule out any non-orthopaedic causes of pelvic limb pain or stiffness. Jugular blood samples were obtained and submitted for biochemical and haematological testing in order to exclude diseases which may impact general anaesthesia. Owners were asked to complete clinical metrology instruments, considering their dog's health over the previous seven days.

Dogs which continued within the study then underwent alfaxalone total intravenous anaesthesia (based on the investigations described in paper 4), following acepromazine premedication. Under alfaxalone anaesthesia, radiography of elbow, stifle, coxofemoral, and lumbosacral joints was performed. Following radiography, EMG recording at the cranial tibial muscle was performed to evaluate nociceptive threshold, stimulus-response characteristics, and temporal summation to dermal electrical stimulation of the plantar aspect of the third digit. I remained unaware of the results of the radiographic imaging during the EMG testing procedure.

Given the disappointing results related to evoking DNIC using a cold water stimulus, we undertook a further review of the literature relating to conditioned pain modulation in humans. It was considered that injection of substances such as capsaicin or hypertonic saline may result in pain which continued beyond the duration of anaesthesia, and that this would be inappropriate in client owned animals. A mechanical stimulus offered the advantage of being rapidly terminated, and preliminary experiments were performed using

application of a bulldog clip to my fingers in order to ascertain that the stimulus was painful but tolerable, and that it did not result in any tissue damage when applied for a duration in excess of one minute. A subset of 11 OA and 12 control dogs underwent DNIC evaluation using the mechanical stimulus. A baseline EMG response was established by delivering electrical stimuli at twice the individually determined nociceptive threshold at a rate of 1Hz for 100 seconds. This was repeated three more times at five minute intervals, during the second and third sessions the bulldog clip was applied to the third digit of the thoracic limb contralateral to EMG recording for a duration of 20 seconds.

As in the previous paper, the hierarchical structure of the data relating to repeated NWR stimulations was accounted for by using multilevel general linear modelling, enabling exploration of factors such as age and bodyweight on the experimental data.

Analysis of owner completed metrology instruments, veterinary musculoskeletal assessments and radiographic OA scores demonstrated significant differences between groups, supporting the criteria for group allocation. Significant group level differences in the processing of nociceptive stimuli were identified; animals affected by OA exhibited larger increases in EMG responses to increasing levels of nociceptive stimulation, regardless of NSAID treatment, indicating that OA affected dogs experienced augmented processing of nociceptive signaling, consistent with central sensitisation. A seemingly paradoxical increase in electrical nociceptive threshold was identified in OA dogs compared with the control group, although neither group exhibited different thresholds to OANSAID. However, the control group were also found to be significantly younger than the OA group, therefore,

especially in light of the data from QST research (Sanchis-Mora et al. 2017), it is possible that this finding related to age rather than disease status.

Although small numbers of animals underwent DNIC testing using a mechanical conditioning stimulus, EMG data demonstrated that this conditioning stimulus did evoke endogenous anti-nociception in control animals, and that this was impaired in dogs affected by OA, suggesting that this is one of the mechanisms likely to contribute to the state of central sensitisation in dogs suffering from OA. A potential therapeutic strategy aimed at restoring endogenous nociceptive modulation (Bannister et al., 2015) may therefore have merit in the treatment of canine OA in some cases. Data from the study were presented in two abstracts at the Association of Veterinary Anesthetists Spring Meeting in March 2017. Since the publication of paper 5, three papers investigating CPM in conscious dogs have been published. In the first Ruel et al., (2018) evaluated the effects on mechanical nociceptive threshold of a ischaemic pain, produced by inflation of a blood pressure cuff to supra-systolic pressure, in healthy dogs. In this study dogs, in which testing was deemed clearly feasible, demonstrated an increase in mechanical nociceptive threshold associated with the conditioning stimulus. This effect was not observed in dogs in which feasibility of testing was judged less than ideal. Monteiro et al., (2018), also evaluated CPM by measuring changes in mechanical nociceptive threshold associated with ischaemic pain caused by a blood pressure cuff. In this study 13 dogs affected by appendicular osteosarcoma (OSA) demonstrated less efficacious CPM compared to seven healthy controls, however no age matching of groups was attempted. All of the control dogs were less than 3 years of age, whilst the OSA group had a mean age of approximately 7 and a half years. In man decreasing CPM efficacy is associated with increasing age (Grashorn et al., 2013), however

once the OSA dogs were treated with cimicoxib their CPM normalised, suggesting that the disease, rather than age was responsible for the initial group differences. Chiu et al., (2020) evaluated CPM by measuring changes in thermal and mechanical threshold associated with heterotopic mechanical nociceptive stimulation in 11 dogs affected by OA, compared with 13 healthy controls. Compared with the control group, the OA group were significantly older. However, the logistic regression model indicated that OA status was a significant predictor of CPM efficacy, whilst age was not significant in the model. The potential benefits of assessing CPM in conscious dogs are that it may be more readily performed in clinical environments and obviates the need for anaesthesia. Potential drawbacks include ethical concerns over inducing pain of sufficient magnitude to elicit CPM, feasibility, and potential cortical modulation of both nociceptive threshold (stress induced analgesia; anxiety induced hyperalgesia (Rhudy & Meagher, 2000) and behavioural responses, which may confound these evaluations.

Research Paper

PAIN®

Electrophysiological characterisation of central sensitisation in canine spontaneous osteoarthritis

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Abstract

In man, central sensitisation (CS) contributes to the pain of osteoarthritis (OA). Dogs with spontaneous OA may also exhibit CS. Electrophysiological reflex measurements are more objective than behavioural assessments and can be used to evaluate CS in preclinical and clinical studies. It was hypothesised that dogs suffering from OA would exhibit electrophysiological characteristics indicative of CS, associated with reduced diffuse noxious inhibitory controls (DNICs). One hundred and seventeen client-owned dogs were recruited to the study. Hind limb nociceptive withdrawal reflex thresholds, stimulus response, and temporal summation characteristics were recorded, during alfaxalone anaesthesia, from 46 OA dogs, 29 OA dogs receiving nonsteroidal anti-inflammatory drugs (OANSAIDs), and 27 breed- and weight-matched control dogs. Efficacy of DNIC was evaluated in 12 control and 11 of the OA dogs, by application of a mechanical conditioning stimulus to the contralateral forelimb. Nociceptive withdrawal reflex thresholds were higher in OA compared with control dogs ($P = 0.02$). Stimulus response characteristics demonstrated an augmented response in OANSAID dogs compared with OA ($P < 0.001$) and control ($P < 0.001$) dogs. Temporal summation demonstrated exaggerated C-fibre-mediated responses in both OA ($P < 0.001$) and OANSAID ($P = 0.005$) groups, compared with control animals. Conditioning stimulus application resulted in inhibition of test reflex responses in both OA and control animals ($P < 0.001$); control animals demonstrated greater inhibition compared with OA ($P = 0.0499$). These data provide evidence of neurophysiological changes consistent with CS in dogs with spontaneous OA and demonstrate that canine OA is associated with reduced DNIC.

Keywords: Central sensitisation, Diffuse noxious inhibitory controls, Dog, Nociceptive withdrawal reflex, Osteoarthritis

1. Introduction

Spontaneous canine osteoarthritis (OA) has been proposed as a model of human OA.[38] In man, in addition to mechanisms local to affected joints, central sensitisation (CS) may exacerbate pain.[29] Some dogs affected by OA respond to centrally acting antihyperalgesic drugs[26] and have altered nociceptive thresholds,[21] suggesting CS; however, there is no "gold standard" approach for identifying and quantifying CS in dogs. Therefore, it is currently unknown whether OA in dogs is also associated with CS, yet this information is essential if canine OA is to be used as a valid model of human OA.

The RIII withdrawal response threshold and magnitude, and temporal summation (TS) to repeated stimuli are altered in pain syndromes associated with CS in man and may be used as objective markers of CS.[37] In dogs, the nociceptive withdrawal reflex (NWR)[6] and TS of the NWR[8] have been suggested as potential biomarkers for CS. We have previously developed methods to evaluate these measures during anaesthesia.[19] There are, presently, no reports of alterations in NWR or TS associated with painful disease in dogs, and the potential for the technique to characterise the state of spinal excitability remains untested.

Diffuse noxious inhibitory controls (DNIC) represent an endogenous supraspinal antinociceptive mechanism activated by heterotopic noxious ("conditioning") stimulation.[5,15] Efficacy of conditioned pain modulation (CPM) in man (considered the equivalent of DNIC) is a predictor of acute[14] and chronic postoperative[41] pain, and is commonly reduced in chronic pain states, including OA.[2] There are no investigations of DNIC efficacy associated with OA in dogs. Conditioned pain modulation may be modulated by cognitive influences,[30] which are challenging to control for experimentally. Therefore, it is desirable to develop a non–tissue-damaging paradigm, which may be applied to anaesthetised animals.

The primary aim of the studies described here was to compare electrophysiological responses, including TS of C-fibre responses, in a cohort of client-owned pet dogs suffering from spontaneous OA, with a matched group of control pet dogs. Dogs within the OA cohort were divided into those receiving daily nonsteroidal

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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anti-inflammatory drugs (NSAIDs) to manage OA-associated pain (OA dogs receiving nonsteroidal anti-inflammatory drugs [OANSAIDs]) and dogs not receiving drug treatment (OA). We hypothesised that dogs with OA would exhibit electrophysiological characteristics indicative of CS, and that these characteristics would be exaggerated in the OANSAID group compared with the OA group because of the greater pain that was likely experienced by OANSAID dogs, despite ongoing NSAID administration.

Our second aim was to develop an effective protocol to evaluate DNIC in dogs. Conditioned pain modulation has been elicited by mechanical conditioning stimulation (MCS)[33]; therefore, we sought to investigate whether MCS would evoke DNIC, and whether DNIC efficacy was decreased by OA. We hypothesised that in control dogs, application of MCS would inhibit the NWR, and that the degree of inhibition would be reduced in dogs affected by OA.

2. Methods

2.1. Nociceptive withdrawal reflex/temporal summation investigation

2.1.1. Ethics

The study was conducted under the terms of the Animal (Scientific Procedures) Act, 1986 (as amended, 2013) (A(SP)A) licence number PPL 30/3157, and the experimental protocol was approved by the University of Bristol Animal Welfare and Ethical Review Body.

2.1.2. Recruitment criteria

Advertisements to recruit participants for the study were posted on social media (Facebook and Twitter), within the local University of Bristol intranet, and within local veterinary practices. For the OA group, suitable dogs were 12 kg body weight and above, of any age, body condition and sex exhibiting suspected painful unilateral or bilateral coxofemoral or stifle degenerative joint disease as evidenced by lameness/stiffness/difficulty in rising or ascending steps. Dogs with primarily forelimb lameness were excluded. During the study recruitment phase, a large proportion of dogs screened were already receiving daily treatment with NSAIDs for musculoskeletal pain, and the decision was made to recruit these animals and permit them to continue daily NSAID treatment, but to designate them as a separate group (OANSAID) for analysis within the study. This decision was based on the fact that pain and disability were still present in these individuals, despite treatment with the NSAID.

The inclusion criteria for the control group were based on the demographics of a cohort of OA dogs recruited to a separate study at the University of Bristol,[16] where a mean (SD) age of 9.5 ± 3 years and weight of 27.5 ± 11.6 kg were recorded. For this study, dogs were recruited to the control group that were 6 years old or

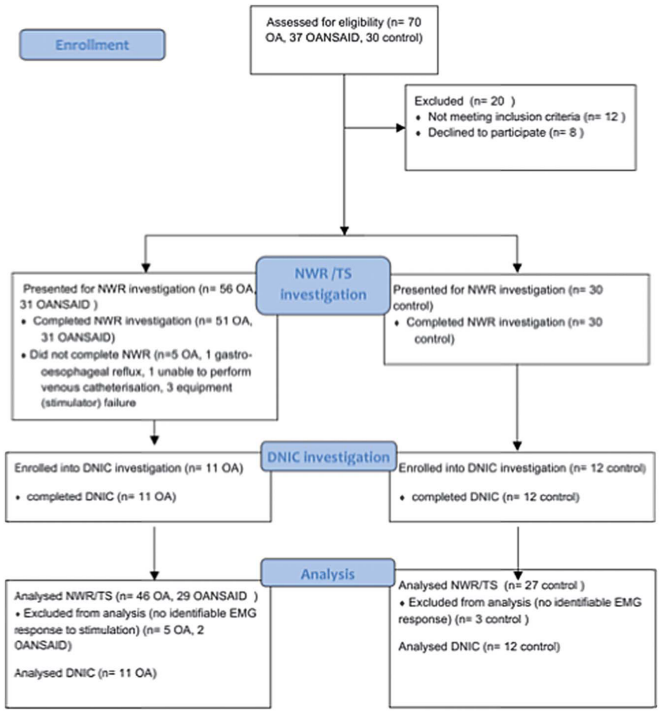

Figure 1. Illustration of the number of animals recruited to each OA category and attrition through different stages of the study. DNIC, diffuse noxious inhibitory control; EMG, electromyography; NWR, nociceptive withdrawal reflex; OA, osteoarthritis; OANSAID, OA dogs receiving nonsteroidal anti-inflammatory drug; TS, temporal summation.

greater and 12 kg body weight and above, exhibiting no evidence of lameness or stiffness and with no other painful condition (eg, otitis externa) and no previous diagnosis of OA. **Figure 1** illustrates the outcomes for all dogs that attended screening and the subsequent numbers that were used at each stage of the study.

2.1.3. Study protocol

Owners of eligible dogs were asked to attend a screening appointment, at which the purpose and procedures of the study were explained verbally and in writing, and signed consent to participate was obtained before any study procedures being performed. Dogs underwent physical and musculoskeletal examination by a veterinarian (J.R.H.). Body condition score (1, emaciated—9, morbidly obese[24]) was assessed by manual palpation. Any dogs with identifiable comorbidities that would have increased risks associated with general anaesthesia, or dogs with neurological dysfunction evidenced by weak or absent conscious proprioception, were excluded from the study. Microchip details were confirmed as a means of permanently identifying participating dogs to comply with the terms of the A (SP)A. Owners were asked to complete the ACVS Canine Orthopaedic Index,[9] the Helsinki Chronic Pain Index,[17] the Liverpool Osteoarthritis in Dogs (LOAD) questionnaire, the Canine Brief Pain Inventory (CBPI),[10] and the sleep and night time restlessness evaluation (SNoRE).[22] Jugular blood samples were obtained and submitted for routine biochemistry and haematology before scheduling general anaesthesia.

2.1.4. Musculoskeletal examination

Scores for lameness (0-10) and mobility (0-3) were assigned by a veterinarian (J.R.H.), according to the criteria shown in Appendix 1 (available online as supplemental digital content at http://links.lww.com/PAIN/A622).

Examination of each joint was performed, and individual appendicular joints were scored from 0 (not affected) to 3 (severely affected) for the criteria "range of motion," "pain on extension or flexion," "crepitus," "effusion," and "thickening." The sum of the joint disease scores produced an overall OA score between 0 and 192, whereas the sum of the pain scores for each joint produced an overall joint pain score between 0 and 48.

2.1.5. General anaesthesia

Seven days after the initial screening appointment, dogs were admitted to the Wellcome Comparative Anaesthesia Research Laboratory, Langford, Bristol, to undergo radiography and NWR testing under general anaesthesia.

On admission, confirmation that dogs had had food withheld for 8 hours was sought from owners and a veterinary examination was repeated.

Acepromazine (ACP 2 mg/mL solution; Elanco Animal Health, Basingstoke, United Kingdom) was administered intramuscularly (0.03 mg/kg), and dogs were left undisturbed for 30 minutes, following which a cephalic venous catheter was placed. Insufficient sedation to permit intravenous catheterisation warranted exclusion from the study.

Alfaxalone (Alfaxan; Jurox [UK] Ltd, Crawley, United Kingdom) (1-2 mg/kg) was administered intravenously over a period of 60 seconds until orotracheal intubation was possible. Oxygen was delivered through a circle breathing system and anaesthesia maintained with a constant rate infusion of alfaxalone (0.1 mg/kg/min) during radiography, reducing to 0.09 mg/kg/min for NWR testing. Body temperature was monitored every 30 minutes and supported with insulated electric blankets. After NWR testing, alfaxalone infusion was discontinued and the dogs constantly monitored until they were discharged to the owner once able to walk and having eaten. All dogs not ordinarily receiving NSAIDs were treated with meloxicam (Metacam 5 mg/mL solution; Boehringer Ingelheim, Bracknell, United Kingdom) (0.2 mg/kg) to treat any pain caused by positioning for radiography or NWR recording.

2.1.6. Radiography

Lateral and cranial–caudal views of the elbows and stifles; lateral views of the lumbosacral junction; and ventrodorsal views of the pelvis and coxofemoral joints were obtained in the Bristol Veterinary School imaging suite. Each of these 7 joints was assessed for severity of radiographic OA by 2 investigators (M.E. and B.D.X.L.) who were unaware of the OA group classification of the dogs. The investigators assigned scores from 0 (no radiographic signs of OA) to 10 (severe radiographic OA) for each joint, and thus, a global score for each dog out of 70 was recorded. The investigators performing NWR testing remained unaware of the results of the radiographs.

2.1.7. Nociceptive withdrawal reflex testing

Dogs were positioned in left lateral recumbency with the right pelvic limb resting on a sandbag, perpendicular to the table top. Paired stimulating electrodes (disposable subdermal needle electrode 12 × 0.40 mm; Natus Neurology Inc, Middleton, WI) were placed 10 mm apart subdermally into the plantar aspect of digit 3 of the right hind limb; paired recording electrodes were placed 20 mm apart into the body of the right cranial tibial (CT) muscle; and a ground electrode placed subcutaneously dorsal to the dorsal spinous process of L6. As previously described,[23] the recorded signal was processed through a differential amplifier (DAM50; World Precision Instruments, Herts, United Kingdom), which applied a band-pass filter from 10 to 1 kHz and gain of 1000, and was subsequently captured in Labchart 8 software (AD Instruments, Oxford, United Kingdom) following conversion by an analogue to digital converter with a sampling frequency of 1 kHz (Powerlab 4/35; AD instruments).

2.1.8. Electromyographic threshold

Electrical stimuli were delivered through the toe electrodes using a constant current stimulator from an isolated 100 V source (Stimulus isolator FE180; AD instruments).

The threshold current at which a single 1-ms square wave stimulating pulse would evoke a visually discernable CT electromyographic (EMG) response (a response greater than the baseline amplitude) was identified by increasing current stepwise from 0 to a maximum of 10 mA in 0.5-mA increments. After a response, the current was decreased by 0.1 mA increments until the response was no longer elicited. This up and down adjustment was continued until 3 stable readings for threshold were obtained at 60-second intervals.

2.1.9. Stimulus response curve

One stimulus event comprised five 1-ms stimuli (Train-of-5 [To5][23]), which were delivered at a frequency of 100 Hz. An EMG stimulus response determination was performed by triggering To5 events at 60-second intervals using currents of 0.1 (baseline), 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mA. The complete series of stimulating currents was

applied in the same ascending order on a second occasion after a 5-minute interval.

2.1.10. Temporal summation

A stimulus sequence of 8 × 1 ms 10-mA stimuli delivered at a rate of 1 Hz was repeated 3 times at 5-minute intervals.

2.1.11. Electromyographic analysis

After recording, a 10-Hz high-pass digital filter was applied to the EMG traces, to further decrease movement artefact. The primary outcome measure for the study was the integral of the rectified EMG response that was extracted for each stimulus within each predefined time window. The EMG response was designated as early (representing an A-fibre response 0-100 ms) or late (C fibre; 100-500 ms) latency, time locked to the start of the stimulus train.[23] Although the late response may also contain components of supraspinal origin, this differentiation was based on previous work in dogs[8] where conduction velocity of the different nerve fiber types and the length of the afferent distance were used to calculate latency ranges for the different (A and C fiber) responses. Baseline activity in the absence of any electrically evoked response (the 0.1-mA stimulus for the stimulus response experiments and from within a 2-second period before application of the first of the 8 stimuli for TS experiments) was subtracted from each measurement.

2.1.12. Statistical methods

Recordings of NWR data were visually examined by 2 investigators (J.R.H. and J.H.) and where no identifiable response could be appreciated to a stimulation protocol, the data for that protocol for the individual dog were excluded from the analysis. Sex distribution data were analysed using χ^2 tests. Comparisons of mean or median measures at single time points (eg, body weight, lameness, and owner-completed metrology instruments) between the 3 groups were performed using 1-way analysis of variance or Kruskal–Wallis tests followed by the Tukey (or Dunn) post hoc testing if applicable. The hierarchical structure of the data comprising the stimulus response and TS data was accounted for by using multilevel modelling within the MLwiN statistics package.[34] In the case of the stimulus response data, no transformation of the outcome variable was necessary, as the residuals from the analyses showed appropriate normality and homoscedasticity. It was necessary to apply natural log transformation to the TS data to meet the assumptions of the statistical models. Data analysed using parametric tests are presented as mean (95% confidence interval [CI]), and the results of nonparametric testing are presented as median (25%-75% interquartile range). The final multilevel general linear models took the form of equations that described the effect of the statistically significant predictor variables on the outcome measures. The parameter estimates from the analyses are presented below, and the models are represented as graphs.

2.1.13. Power calculation

A power calculation for the overarching project, based on preliminary data using von Frey mechanical threshold data, indicated a total of 68 dogs, evenly divided between OA and control groups, would be required for a power of 90%, at an alpha of 0.05 to detect a difference between control and OA dogs. However, this calculation assumed uniformity within the OA group, whereas we suspected that the OA group would be heterogenous, based on data from human OA patients and laboratory animal models of OA. In humans, up to 70% of patients with OA have at least one somatosensory abnormality.[40] Based on this, we estimated that recruiting 100 OA dogs would give us an appropriate cohort of CS-negative dogs (ie, approximately the same number as control dogs) and a cohort of CS-positive dogs that may be as large as 70.

2.2. Diffuse noxious inhibitory control investigation

2.2.1. Animals

After completion of the NWR/TS protocol described above, some dogs underwent DNIC investigations during the same anaesthetic period (**Fig. 1**).

2.2.2. Diffuse noxious inhibitory control protocol

Five minutes after the final TS experiment, the DNIC protocol began by recording EMG responses in the CT muscle to test stimuli delivered at twice the individually determined threshold current (2xThr) at a rate of 1 Hz for 100 seconds. This occasion was denoted as "pre-DNIC." An identical test stimulation protocol (2xThr, 1 Hz, 100 seconds) was repeated on 3 more occasions at 5-minute intervals; however, during occasions 2 ("DNIC 1") and 3 ("DNIC 2"), the effect on CT responses of an additional mechanical conditioning stimulus, comprising a bulldog clip applied for 20 seconds to the third digit of the contralateral forelimb, was assessed (**Fig. 2**). The fourth and final occasion ("post-DNIC") was a repeat of the pre-DNIC stimulating protocol, without the addition of a conditioning stimulus.

Measurement of the force delivered by the bulldog clip at a jaw separation of 11 mm (mean jaw opening measured during the application to the digit) was achieved using a Loadcell 50 N gauge (Mecmesin; Slinfold, West Sussex, United Kingdom). The force recorded by the gauge at 11-mm separation was 33.4 N, but this was also found to be consistent over the range of jaw opening from 2 to 12 mm. Examination of the site of application following the DNIC protocol, and 7 days later, demonstrated no evidence of immediate or delayed ongoing pain or tissue damage.

2.2.3. Statistical methods

Sex distribution data were assessed using the Fisher exact test. Comparisons of weight and owner-completed metrology instrument scores between the 2 groups were performed using the Student *t* test or Mann–Whitney *U* test. The hierarchical structure of the DNIC testing data was accounted for within the statistical analysis by using general linear modelling within a multilevel modelling framework using the MLwiN statistics package.[41] Predictor variables were retained within the model based on a Wald test at $\alpha \leq 0.05$. It was necessary to apply a natural log transformation to the EMG magnitude data, to meet the assumptions of the tests regarding normality and homoscedasticity of residuals. The pre-DNIC occasion was denoted as the reference occasion for comparisons within the model. Data subject to parametric tests are presented as mean (95% CI) and results subject to nonparametric testing are presented as median (25%-75% interquartile range).

2.2.4. Power calculation

A power calculation was performed for the overarching project; however, the DNIC investigation was performed to develop an effective but non–tissue-damaging model for evaluating DNIC in

Figure 2. During anaesthesia, a bulldog clip conditioning stimulus was applied for 20 seconds to the third digit of the left cranial limb, whereas electrical test stimuli were delivered to the right pelvic limb.

dogs, and to provide pilot data for ongoing investigations, hence a power calculation was not performed specifically regarding the primary outcome measure (magnitude of EMG response) reported here.

3. Results

3.1. Nociceptive withdrawal reflex/temporal summation investigation

3.1.1. Demographics

Data were analysed from 27 control, 46 OA, and 29 OANSAID dogs. Breed and sex distribution are shown in **Table 1**. There was no significant difference in sex distribution, and breed distribution appeared to be visually well matched between groups. Weight and body condition scores were not different between groups (**Table 1**). Dogs in the control group were younger than dogs in both the OA and OANSAID groups (**Table 1**). The duration of NSAID treatment in the OANSAID group was variable between individuals, but animals had been receiving daily NSAIDs for at least 3 months before recruitment to the study.

3.1.2. Veterinary assessment

Degree of lameness, mobility score, total OA score, and total joint pain score were all significantly higher in OA and OANSAID groups compared with controls (**Table 2**); however, there were no differences between OA and OANSAID groups regarding these measures.

Table 1
Demographic data.

	Control (n = 27)	OA (n = 46)	OANSAID (n = 29)	*P*
Breed				
Border collie	7	10	5	—
Labrador	5	8	11	—
Retriever	3	3	1	—
Lurcher	3	2	0	—
Spaniel	1	5	3	—
Other	8	18	9	—
Sex				
M	3	3	3	0.61
Mn	7	18	14	0.61
F	1	3	2	0.61
Fn	16	22	10	0.61
Weight (kg)	22.8 (95% CI 20.5-25.0)	26.8 (95% CI 23.6-29.9)	28.7 (95% CI 24.8-32.6)	0.0563
Body condition score (1-9)	5 (4-6)	5 (5-6)	5 (4-6)	0.19
Age (y)	7.8 (95% CI 7.3-8.4)[a]	9.8 (95% CI 9.2-10.3)[b]	9.6 (95% CI 8.5-10.6)[b]	<0.001***

Superscript letters indicate groupings within the data, shared superscripts indicate no significant difference between groups on post hoc testing, and differing superscripts indicate a difference with a *P* value of less than 0.05 on post hoc testing. ***$P \leq 0.001$.
F, female; Fn, female neuter; M, male; Mn, male neuter; OA, osteoarthritis; OANSAID, OA dogs receiving nonsteroidal anti-inflammatory drug.

3.1.3. Owner-completed clinical metrology instruments

Questionnaire data were analysed by subsection if the questionnaire was constructed in a section format. Owner-attributed scores for all the questionnaire subsections were significantly higher (more dysfunction/pain) in OA and OANSAID animals compared with controls. In addition, the CBPI pain and ACVS function subsections were significantly higher in OANSAID compared with OA animals (**Table 2**), indicating that dogs receiving NSAID therapy experienced greater pain and greater dysfunction (eg, reduced mobility) than dogs with OA that were not receiving NSAID treatment.

3.1.4. Radiographic scores

Radiographic OA severity was significantly higher in both OA and OANSAID animals compared with controls but was not significantly different between OA and OANSAID animals (**Table 2**).

3.1.5. Nociceptive withdrawal reflex recordings

The early phase of the NWR could be reliably and repeatedly elicited in the CT muscle during the multiple trials at each stimulus intensity. Examples of raw traces obtained during NWR recording are provided (**Figs. 3 and 4**).

3.1.6. Electrical threshold

The threshold current to elicit an EMG response was significantly lower in control (2.3 [95% CI 1.8-2.9 mA]) compared with OA dogs (3.8 [95% CI 3.0-4.6 mA] [$F_{2,93} = 3.859$, $P = 0.02$]), but neither group was different from OANSAID (3.2 [95% CI 2.4-3.9 mA]) that had an intermediate value.

3.1.7. Stimulus response

Only the early component of the response was analysed, as the late response was absent in the majority of recordings (**Table 3**).

Table 2

Musculoskeletal examination, owner-completed metrology instrument, and radiographic severity data.

	Control	OA	OANSAID	*P*
Lameness (0-10)	0 (0-0)[a]	3 (1-3)[b]	3 (2-3)[b,c]	<0.001***
Mobility (0-3)	0 (0-0)[a]	1 (1-1)[b]	1 (1-1)[b,c]	<0.001***
OA score (0-192)	0 (0-2)[a]	10 (7-16)[b]	14 (9-19)[b,c]	<0.001***
Joint pain score (0-48)	0 (0-0)[a]	4 (2-4)[b]	4 (3-5)[b,c]	<0.001***
CBPI pain (0-10)	0 (0-0.0625)[a]	1.75 (0-3.5)[b]	3.375 (1.813-4.688)[c]	<0.001***
CBPI function (0-10)	0 (0-0.833)[a]	1.167 (0.1667-4.50)[b]	2.833 (1.50-5.042)[b,c]	<0.001***
HCPI (0-44)	3 (0-8.25)[a]	14 (8-22)[b]	20.5 (15.25-21.75)[b,c]	<0.001***
ACVS stiffness (0-16)	0 (0-0.25)[a]	5 (2-8)[b]	8 (5-9)[b,c]	<0.001***
ACVS function (0-16)	0 (0-0.25)[a]	5 (1-8)[b]	8 (6-12)[c]	<0.001***
ACVS gait (0-20)	0.5 (0-2.25)[a]	7 (2-11)[b]	9 (7-11.75)[b,c]	<0.001***
ACVS QoL (0-12)	0 (0-1)[a]	3 (1-5)[b]	4.5 (2.6)[b,c]	<0.001***
LOAD (0-52)	2 (0-5)[a]	14 (9-23)[b]	18.5 (12-23)[b,c]	<0.001***
Radiographic OA score (0-70)	3 (1-10)[a]	14 (8.25-24.75)[b]	20 (8-26)[b,c]	<0.001***

Superscript letters indicate groupings within the data, shared superscripts indicate no significant difference between groups on post hoc testing, and differing superscripts indicate a difference with a *P* value of less than 0.05 on post hoc testing. ***$P \leq 0.001$.
CBPI, Canine Brief Pain Inventory; HCPI, Helsinki Chronic Pain Index; LOAD, Liverpool Osteoarthritis in Dogs; OA, osteoarthritis; OANSAID, OA dogs receiving nonsteroidal anti-inflammatory drug.

The parameter estimates of those predictor variables significantly associated with the response are presented in **Table 3**. The final model, containing only the significant terms, demonstrated that the magnitude of the measured response increased as a curvilinear function of the stimulating current (mA). There was a significant negative interaction between body weight and stimulating current (weight.mA; $P < 0.001$); larger animals demonstrated a lesser increase in response magnitude with increasing current compared with smaller animals. There was a significant positive interaction between the OANSAID category and stimulating current (OANSAID.mA) compared with control ($P < 0.001$) and OA category ($P < 0.001$) animals; OANSAID category animals demonstrated increased magnitude responses at a given stimulating current, compared with both control and OA category animals. These relationships are shown graphically in **Figure 5**, at a fixed weight of 25 kg.

3.1.8. Temporal summation early (A-fibre) response

The magnitude of A-fibre responses increased with increasing stimulus number from 1 to 8 within each repetition of the protocol (TS) ($P < 0.001$) but was reduced on the third (final) occasion of the TS (train of 8) protocol, compared with the first ($P = 0.013$) (**Table 4**). Higher weight animals demonstrated reduced magnitude responses to stimulation ($P = 0.001$) and lesser increases in magnitude of response with increasing stimulus number (weight. stimulus number interaction) ($P = 0.009$). Osteoarthritis and OANSAID animals did not differ from control animals.

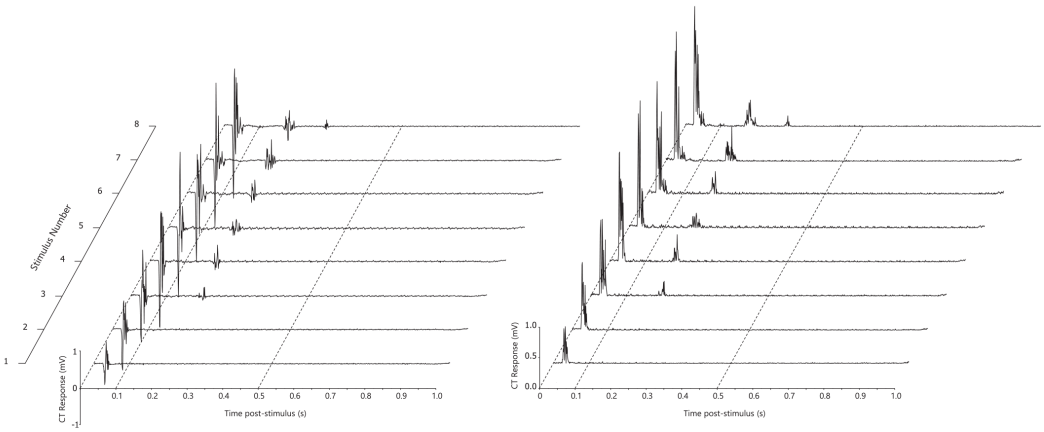

Figure 3. An example of temporal summation in the cranial tibial muscle (recorded from dog 71). The top channel is a stimulus marker channel, with a train of 8, 1-ms 10-mA stimuli delivered at a frequency of 1 Hz. The lower channel shows the early and late responses in the cranial tibial muscle. The time base is 0.2 seconds/division. CT, cranial tibial.

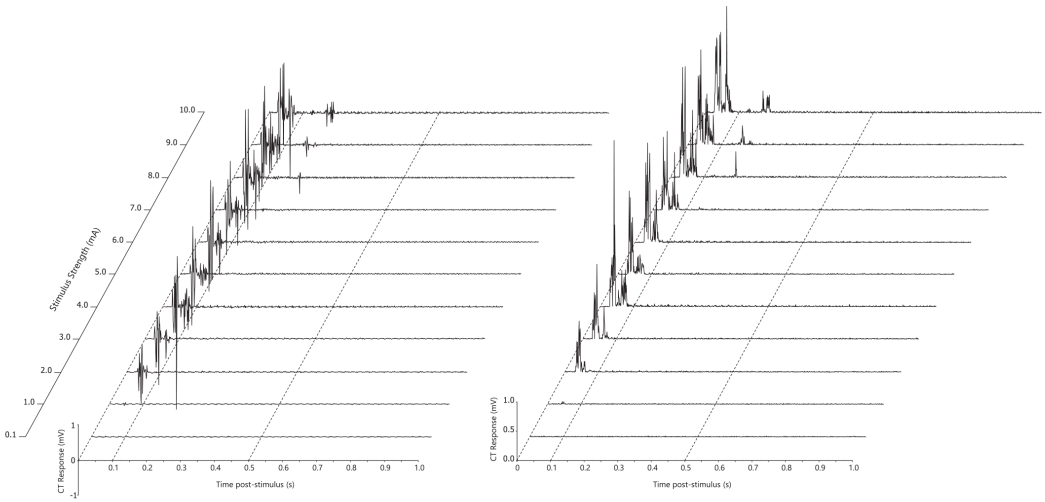

Figure 4. An example of the electrical stimulus response curve recorded from the cranial tibial muscle in dog 98. The top channel is the stimulus marker channel, with each single line representing five 1-ms stimuli delivered at a frequency of 100 Hz. Eleven stimuli were delivered with a 60-second interval between them starting at 0.1 mA (baseline), 1 mA, and increasing in 1 mA increments through to 10 mA. The middle channel shows the early responses in the cranial tibial muscle, and the lower channel shows the rectified EMG response in the cranial tibial muscle. The time base is 0.2 seconds/division. CT, cranial tibial; EMG, electromyography.

3.1.9. Temporal summation late (C-fibre) response

The TS protocol consistently elicited late responses (**Table 4**). The magnitude of the late (C-fibre) response increased with increasing stimulus number from 1 to 8 within each repetition of the protocol (TS) ($P < 0.001$) but was decreased on both the second and third occasions of repeating the protocol (train of 8) compared with the first trial. Higher weight animals demonstrated lesser increases in magnitude of response with increasing stimulus number (weight.stimulus number interaction; $P < 0.001$), and older animals also demonstrated lesser increases in magnitude of response with increasing stimulus number (age. stimulus number interaction; $P = 0.001$). Both OA (OA.stimulus number interaction; $P < 0.001$) and OANSAID (OANSAID. stimulus number interaction; $P = 0.005$) category animals demonstrated larger increases in magnitude of response with increasing stimulus number compared with control animals (**Fig. 6**), but there were no differences between the OA and OANSAID groups.

3.2. Diffuse noxious inhibitory control investigation

3.2.1. Demographics

Data were analysed from 12 control and 11 OA dogs (none receiving NSAIDs). The sex distribution between the groups was not different, and the distribution of breeds appeared well matched on visual inspection (**Table 5**). Osteoarthritis dogs were significantly older than control dogs (**Table 5**). Groups were not different in terms of weight; however, body condition score was

Table 3

Effect size estimates and *P* values for the general linear model that was fitted to the stimulus response (early) data.

	Response magnitude (mV/s)	SE	Conf int 2.5%	Conf int 97.5%	*P*
Intercept	−0.001230	0.003234	−0.007569	0.005109	0.704
Weight	0.000018	0.000113	−0.000204	0.000240	0.873
OA	0.000753	0.002481	−0.004110	0.005615	0.762
OANSAID	0.000353	0.002782	−0.005100	0.005806	0.899
mA	0.004864	0.000540	0.003807	0.005922	<0.001***
mA^2	−0.000170	0.000052	−0.000271	−0.000069	0.001**
weight.mA	−0.000094	0.000019	−0.000132	−0.000056	<0.001***
weight.mA^2	0.000004	0.000002	0.000001	0.000008	0.026*
OA.mA	−0.000092	0.000119	−0.000325	0.000141	0.440
OANSAID.mA	0.000759	0.000134	0.000497	0.001021	<0.001***

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
OA, osteoarthritis; OANSAID, OA dogs receiving nonsteroidal anti-inflammatory drug.

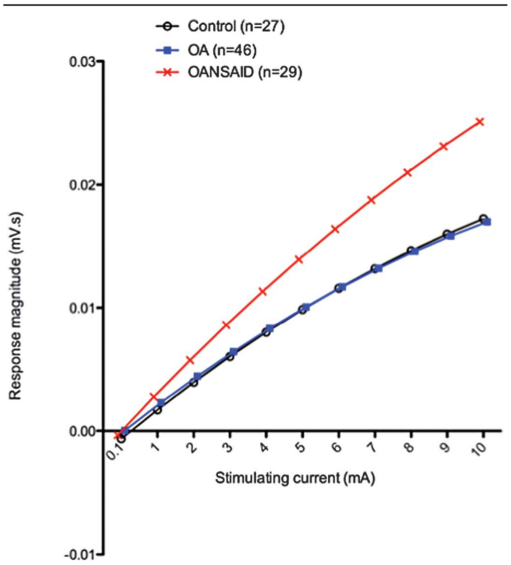

Figure 5. Illustration of the mean curves predicted by the general linear model for stimulus response of dogs within differing OA categories, assuming a weight of 25 kg. Each data point for the control animals is based on 27 dogs; for the OA group, it is based on 46 dogs; and for the OANSAID group, it is based on 29 dogs. For each animal, the mean response to the 2 repetitions of the stimulus response curve was averaged before analysis. The y-axis represents the natural logarithm of the magnitude of the EMG response, and the x-axis shows the magnitude of the stimulating current. EMG, electromyography; OA, osteoarthritis; OANSAID, OA dogs receiving nonsteroidal anti-inflammatory drug.

higher in OA (6, 5-7) compared with control dogs (5, 4.25-5.75, $P = 0.047$).

3.2.2. Veterinary musculoskeletal and gait assessments

Degree of lameness, mobility impairment, OA burden, and joint pain burden were all increased in OA compared with control dogs (**Table 6**).

3.2.3. Owner-completed clinical metrology instruments

The CBPI, Helsinki Chronic Pain Index, ACVS Canine Orthopaedic Index, and LOAD were all rated significantly higher by owners of OA compared with control dogs (**Table 6**), but there was no significant difference in scores for the SNoRE questionnaire.

3.2.4. Radiography

Significantly more radiographic signs of OA were identified in dogs in the OA compared with control group, and significantly more of the 7 joints assessed demonstrated radiographic signs of OA in OA compared with control dogs (**Table 6**).

3.2.5. Nociceptive withdrawal reflex threshold

The threshold current required to elicit an NWR was significantly higher in OA (3.8 [95% CI 2.4-5.2 mA]) compared with control dogs (1.9 [95% CI 1.4-2.5 mA], $P = 0.013$) (**Table 6**).

3.2.6. Diffuse noxious inhibitory control efficacy

The 2xThr stimulation did not elicit consistent late responses, therefore only the early (0-100 ms) latency response was analysed.[19]

The final significant general linear model that described the magnitude of the early response took the form of an equation, the parameter estimates of which and P values associated with the predictor variables within the model are presented in **Table 7**. The predictor variables and their relationship with the magnitude of the response are described below. Time and age were considered continuous scale variables. Each occasion of DNIC testing (pre, DNIC 1, DNIC 2, and post) was considered a categorical variable, as was OA status (OA/control). **Figure 7** shows the effect of mechanical "conditioning" stimulation of the forepaw on electrically evoked "test" EMG reflexes in the CT muscle of the contralateral hind limb.

3.2.6.1. Stability of response magnitude within occasion

Time alone did not account for a significant variation in magnitude within a test occasion ($P = 0.069$).

3.2.6.2. Stability of response magnitude between occasions

Between different test occasions, response magnitude was decreased in DNICs 1 and 2, and in the post-DNIC state, compared with the original pre-DNIC occasion ($P = 0.048$, <0.001, and <0.001 respectively), indicating a decreasing magnitude of response with repeated occasions of the stimulating protocol.

3.2.6.3. Efficacy of diffuse noxious inhibitory control stimulus

There was a significant interaction between time and occasion for DNICs 1 and 2 ($P <0.001$), but not between time and occasion post-DNIC ($P = 0.50$), demonstrating that the application of the conditioning stimulus was responsible for significantly decreasing the response magnitude during DNICs 1 and 2 compared with the pre-DNIC occasion. The interaction between square and cubic terms of time, and DNICs 1 and 2, was significant, indicating a curvilinear change of response with application of the conditioning stimulus.

3.2.6.4. Effect of osteoarthritis status

Osteoarthritis status alone had no significant effect on response magnitude ($P = 0.31$); however, there was a significant interaction between OA status and occasion during the DNIC 2 ($P = 0.003$) and post-DNIC ($P = 0.02$) testing, which predicted a higher magnitude of response (ie, decreased inhibition of response) in OA dogs during these 2 occasions, compared with control dogs. Inclusion of the overall interaction between OA status and DNIC occasion as a predictor variable significantly improved the model (change in log likelihood = 7.82, df3; $P = 0.0499$).

3.2.6.5. Effect of age

The effect of age was tested within models but found to be not significant as either a main effect within the model, nor in interaction with other terms within the models.

4. Discussion

These studies have shown that several characteristics of the CT NWR were altered in dogs with OA, therefore central neurophysiological changes may play a role in the pathology

Table 4

Effect size estimates and *P* values for the general linear model that was fitted to the temporal summation data.

	Temporal summation early response (lnmV/s)	SE	Conf int 2.5%	Conf int 97.5%	*P*	Temporal summation late response (lnmV/s)	SE	Conf int 2.5%	Conf int 97.5%	*P*
Intercept	−4.900	0.341	−5.569	−4.231	<0.001***	−7.142	0.700	−8.513	−5.771	<0.001*
Weight	−0.040	0.012	−0.064	−0.017	0.001**	0.017	0.015	−0.012	0.046	0.246
OA						−0.722	0.348	−1.404	−0.040	0.038*
OANSAID						−0.254	0.362	−0.964	0.456	0.483
Occasion 2	−0.022	0.019	−0.059	0.016	0.265	−0.058	0.026	−0.109	−0.007	0.026*
Occasion 3	−0.048	0.019	−0.086	−0.010	0.013*	−0.120	0.026	−0.171	−0.069	<0.001***
Stimulus number	1.084	0.145	0.800	1.369	<0.001***	2.401	0.373	1.670	3.132	<0.001***
Stimulus number^2	−0.243	0.036	−0.314	−0.171	<0.001***	−0.474	0.094	−0.658	−0.291	<0.001***
Stimulus number^3	0.016	0.003	0.011	0.021	<0.001***	0.030	0.007	0.016	0.043	<0.001***
Weight. stimulus number	−0.014	0.005	−0.024	−0.003	0.009**	−0.037	0.008	−0.053	−0.022	<0.001***
Weight. stimulus number^2	0.004	0.001	0.001	0.006	0.006**	0.008	0.002	0.004	0.012	<0.001***
Weight. stimulus number^3	0.000	0.000	0.000	0.000	0.009**	−0.001	0.000	−0.001	0.000	<0.001***
OA.stimulus number			—	—	—	0.805	0.186	0.442	1.169	<0.001***
OANSAID.stimulus number			—	—	—	0.540	0.193	0.161	0.919	0.005**
OA.stimulus number^2			—	—	—	−0.160	0.047	−0.251	−0.069	0.001**
OANSAID.stimulus number^2			—	—	—	−0.100	0.048	−0.195	−0.005	0.039*
OA.stimulus number^3			—	—	—	0.010	0.003	0.004	0.017	0.003**
OANSAID.stimulus number^3			—	—	—	0.006	0.004	−0.001	0.013	0.079
Age			—	—	—	0.088	0.067	−0.043	0.218	0.187
Age.stimulus number			—	—	—	−0.121	0.036	−0.190	−0.051	0.001**
Age.stimulus number^2			—	—	—	0.024	0.009	0.007	0.042	0.006**
Age.stimulus number^3			—	—	—	−0.002	0.001	−0.003	0.000	0.016*

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
OA, osteoarthritis; OANSAID, OA dogs receiving nonsteroidal anti-inflammatory drug.

of OA-associated pain and disability in dogs. Diffuse noxious inhibitory control investigations suggest that these central changes may be related in part to less effective descending inhibition of nociceptive stimuli.

In man, the RIII (Aδ-fibre-mediated) threshold is correlated with the pain threshold[35] and is decreased in painful OA states.[12] We anticipated that dogs exhibiting CS would demonstrate a diminished threshold to elicit an NWR; however, our results indicated that threshold current was higher in OA animals compared with controls. The underlying reason for this finding is difficult to explain. The early latency (0-100 ms) response elicited by NWR stimulation in our testing paradigm comprises both Aβ (RII equivalent in man) and Aδ (RIII equivalent) transmission. The RII response in man is considered non-nociceptive and elicited by subpain threshold intensities of stimulation. Central sensitisation may be accompanied by hypoaesthesia to one or more sensory modalities in human subjects,[18] therefore it is possible that the greater threshold identified in OA dogs relates to Aβ-mediated hypoaesthesia. Although it may have been desirable to further divide the responses by latency into Aβ- or Aδ-mediated, as reported by Bergadano et al.,[6] we undertook testing in a mixed population of dogs with a range of weights and conformations, which would have added to the variability in response latency. Visual inspection of pilot data traces revealed that we could only consistently identify an early (A-fibre) and late (C-fibre) response.[19] We could have considered measuring the afferent distance of the conduction pathway in individual animals and using this, together with an estimate of conduction velocity, to calculate more accurately the latency window of the NWR in each individual dog. However, our inclusion criteria for the study limited the weight range of the dogs included in the study, therefore this was not deemed necessary for the present investigation.

The stimulus response curve demonstrated facilitation of the early response in OANSAID dogs, compared with both control and OA dogs. The amplitude of the RIII response has been shown to correlate with the magnitude of subjective pain in conscious human volunteers[13]; therefore, the inference from our data is that

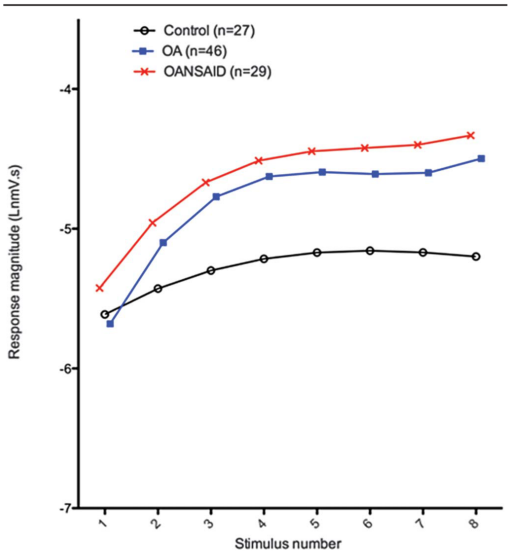

Figure 6. Illustration of the mean curves predicted by the general linear model for the first occasion temporal summation late response for dogs within differing OA categories, assuming a weight of 25 kg and age of 9 years. The y-axis represents the natural logarithm of the magnitude of the EMG response, and the x-axis shows stimulus number. EMG, electromyography; OA, osteoarthritis; OANSAID, OA dogs receiving nonsteroidal anti-inflammatory drug.

OANSAID dogs may exhibit hyperalgesia, compared with dogs in both the OA and control groups. The fact that the OA and OANSAID groups were not different based on veterinarian examination scores and radiographic OA scores were not unexpected—there are no validated veterinarian assessment systems of OA pain, and radiographic evidence of OA is known not to be correlated with pain, just as in humans. Although OA and OANSAID groups were comparable with respect to the majority of the clinical metrology instrument data, OANSAIDs were significantly more affected with respect to the CBPI pain and the ACVS description of function subscales and had higher scores on all the other validated clinical metrology instruments (LOAD and CBPI function). These data indicate that the OANSAID group was more severely affected by OA pain and suggest that treatment with commonly prescribed veterinary NSAIDs[20] may not prevent or reverse CS, despite the tentative conclusion from a recent study in humans with OA investigating etoricoxib.[1] The total duration of treatment with NSAIDs in the OANSAID group was not recorded in individual dogs in this study, and it is possible that differences in the duration of administration introduced variability into the data. However, all dogs in the OANSAID group had been receiving NSAIDs for at least 3 months before recruitment to the study which, from early data in humans,[1] would be sufficient time for the NSAID to exhibit an antihyperalgesic effect.

Table 5
Demographic data.

	Control (n = 12)	OA (n = 11)	*P*
Breed			
Labrador	6	3	—
Collie	2	1	—
Retriever	2	2	—
Lurcher	2	1	—
German Shepherd	0	1	—
Rottweiler	0	2	—
Spaniel	0	1	—
Sex			
Male neuter	6	5	1.0
Female neuter	6	6	1.0
Weight	23.8 (95% CI 21.6-26.1)	31.3 (95% CI 23.2-39.4)	0.053
Age	7.5 (95% CI 6.9-8.2)	9.8 (95% CI 8.5-11.1)	0.002**
Body condition score (0-9)	5 (4.25-5.75)	6 (5-7)	0.047*

*$P \leq 0.05$; **$P \leq 0.01$.
OA, osteoarthritis.

Temporal summation data demonstrated no group differences for the early (A-fibre-mediated) response but facilitation of the late (mostly C-fibre) response in OA and OANSAID dogs, compared with controls. The absence of an effect on the early response data is likely due to a significant component being mediated by low-threshold A-beta fibres. The applied 10-mA stimulus, designed as a suprathreshold stimulus, would cause the early response to saturate at this level of stimulation, and therefore, differences between groups were minimised. By contrast, the higher-threshold C-fibre-mediated late response displayed the expected increasing magnitude with repeated stimuli and, in alignment with our hypothesis, was augmented in both OA and OANSAID groups compared with the control group. This likely indicates that OA is associated with CS in dogs. It is also possible that the EMG findings for C-fibre-mediated responses are due to C-fiber sensitisation rather than CS, although it is difficult to make a distinction between these 2 effects in our data set.

The data produced during the DNIC investigation demonstrate both that MCS elicits quantifiable DNIC in anaesthetised dogs, and that the efficacy of DNIC is compromised in dogs with OA, compared with a control group. A recent meta-analysis concluded that, despite methodological limitations, a number of chronic pain conditions in man, including OA, are associated with reduced efficacy of CPM.[28] Reduced net efficacy of nociceptive inhibition may arise through impaired descending antinociceptive modulation or through descending facilitation of nociceptive signalling.[3] We did not probe each of these pathways independently in these clinical cases; however, the magnitude of measured EMG response in this study represents the net effect of balance between inhibitory and facilitatory mechanisms; therefore, these data provide evidence that the balance of descending pathways becomes shifted toward pronociception in canine OA.

The differences between OA and control groups were only evident on DNIC 2, and then persisted into the post-DNIC period. Because previous data on DNIC in dogs using MCS were not available, numbers required to identify significant differences were unknown; however, it is clear from our results that the interaction between group and occasion begins to approach significance during DNIC 1 ($P = 0.07$). Had larger sample sizes been used, we would have had greater power to detect differences between groups, and may have identified a significant difference during DNIC 1. The small sample size is a major limitation of the DNIC investigation and reflected difficulties in establishing the methodology to elicit DNIC in dogs. Only 5 minutes was allowed to elapse between the TS protocol and the start of the DNIC investigation. This time period was kept deliberately short to avoid prolonging the anaesthesia time for the dogs as far as possible. It is possible that delivery of

Table 6

Musculoskeletal examination, owner-completed metrology instrument, radiographic scoring, and nociceptive withdrawal reflex (NWR) data in dogs undergoing the DNIC protocol.

	Control	OA	*P*
Lameness (0-10)	0 (0-0)	3 (3-3)	<0.001***
Mobility (0-3)	0 (0-0)	1 (1-2)	<0.001***
OA score (0-192)	0 (0-2)	9 (6-12)	<0.001***
Joint pain score (0-48)	0 (0-0)	4 (2-5)	<0.001***
CBPI pain (0-10)	0 (0-0)	1.125 (0-2.69)	0.0085**
CBPI function (0-10)	0 (0-0)	2.375 (0-6.938)	0.0022**
HCPI (0-44)	1 (0-1.75)	15.5 (3.5-20.5)	0.0026**
ACVS stiffness (0-16)	0 (0-0)	5.5 (0-7)	0.0029**
ACVS function (0-16)	0 (0-0)	4 (0-8.75)	0.0076**
ACVS gait (0-20)	0 (0-0)	5 (2.25-11.5)	0.0022**
ACVS QoL (0-12)	0 (0-0.75)	3 (0-6.25)	0.0076**
LOAD (0-52)	2.5 (0-3)	15.5 (5-25)	0.0042**
SNoRE	13.5 (10.5-18.5)	15.5 (14-25.25)	0.21
Radiographic OA score (0-70)	2 (0.25-3)	20 (16-28)	<0.001***
Number of joints radiographically affected	1 (0.25-2)	5 (2-6)	<0.001***
NWR threshold	1.9 (95% CI 1.4-2.5)	3.8 (95% CI 2.4-5.2)	0.013*

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
CBPI, Canine Brief Pain Inventory; CI, confidence interval; DNIC, diffuse noxious inhibitory control; HCPI, Helsinki Chronic Pain Index; LOAD, Liverpool Osteoarthritis in Dogs; OA, osteoarthritis; SNoRE, sleep and night time restlessness evaluation.

Table 7

Parameter estimates, SE, 95% CIs, and *P* values for the general linear model fitted to the stimulus response (early) data (ln(mV/s)).

Predictor variable	Parameter estimate	SE	Conf int 2.5%	Conf int 97.5%	*P*
Fixed effects					
Cons	−5.420132	0.265083	−5.939685	−4.90058	<0.001***
DNIC 1	−0.158861	0.095373	−0.345789	0.028067	0.048*
DNIC 2	−0.508912	0.095373	−0.695839	−0.321984	<0.001***
Post-DNIC	−0.433574	0.100943	−0.631419	−0.235729	<0.001***
Time	−0.009741	0.006579	−0.022635	0.003153	0.069
$Time^2$	−0.000054	0.000418	−0.000873	0.000764	0.448
$Time^3$	0.000005	0.000007	−0.00001	0.000019	0.265
OA	−0.183357	0.381486	−0.931054	0.564341	0.315
OA.DNIC 1	0.181664	0.127397	−0.068029	0.431357	0.077
OA.DNIC 2	0.349945	0.127397	0.100251	0.599638	0.003**
OA.post-DNIC	0.271047	0.131377	0.013553	0.528541	0.020**
Time.DNIC 1	−0.055631	0.009303	−0.073866	−0.037397	<0.001***
$Time^2$.DNIC 1	0.003449	0.000591	0.002291	0.004607	<0.001***
$Time^3$.DNIC 1	−0.000052	0.00001	−0.000072	−0.000032	<0.001***
Time.DNIC 2	−0.05043	0.009303	−0.068664	−0.032195	<0.001***
$Time^2$.DNIC 2	0.00353	0.000591	0.002372	0.004688	<0.001***
$Time^3$.DNIC 2	−0.000057	0.00001	−0.000077	−0.000037	<0.001***
Time.post-DNIC	0.000054	0.009522	−0.01861	0.018717	0.497
$Time^2$.post-DNIC	0.000264	0.000605	−0.000922	0.001449	0.331
$Time^3$.post-DNIC	−0.000006	0.00001	−0.000026	0.000015	0.299

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
CI, confidence interval; DNIC, diffuse noxious inhibitory control; OA, osteoarthritis.

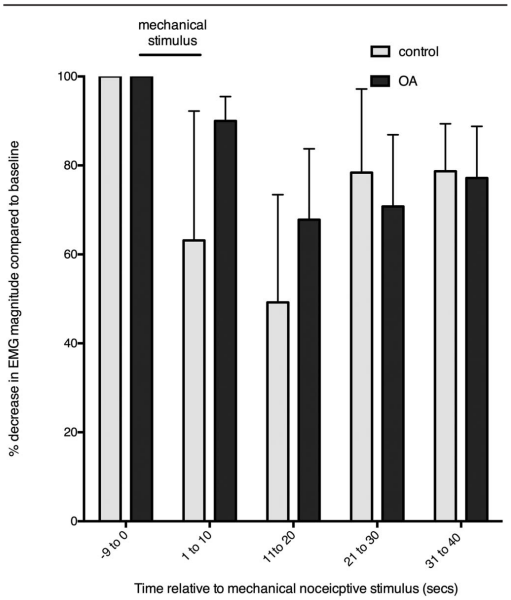

Figure 7. Effect of mechanical "conditioning" stimulation of the forepaw on electrically evoked "test" EMG reflexes in the cranial tibial muscle of the contralateral hind limb. Clip was applied at time 0 for 20 seconds. In the control group, EMG responses to the test stimulus were reduced (greater % reduction in EMG) during clip application, indicating antinociception and a DNIC effect. When all time points were considered, DNIC in the OA group (n = 11) was significantly less compared with control animals (n = 12) (P = 0.016). Responses are medians, errors are 75th percentiles. DNIC, diffuse noxious inhibitory control; EMG, electromyography; OA, osteoarthritis.

a supramaximal stimulus during the TS protocol sensitised the nociceptive system so that the nociceptive pathways were not in a naive state at the start of the DNIC experiment, and this may have affected our DNIC results. The optimal time delay between TS and measurement of DNIC is currently unknown.

Nociceptive withdrawal reflexes are segmental spinal reflexes, subject to supraspinal modulation.[11] Alfaxalone anaesthesia enabled NWR recording in client-owned dogs. While alfaxalone increases NWR threshold and decreases magnitude of response to electrical stimulation,[19] there is no reason to expect a differential effect of the anaesthetic on control vs OA or OANSAID animals, as alfaxalone is devoid of analgesic activity.[39]

Regarding assessment of DNIC, many sedatives and analgesics will interact with descending pronociceptive and antinociceptive pathways[27,36] and could alter the measured responses. Acepromazine has been shown not to modulate NWR[7] and, given it is considered to have no antinociceptive properties,[4] would not be expected to interact with descending modulatory mechanisms. Alfaxalone is a gamma aminobutyric acid agonist, and DNIC is reportedly unaffected by gamma aminobutyric acid agonists,[23] therefore we consider that the form of anaesthesia used was appropriate to our investigation.

Although we have identified group-level differences in DNIC efficacy, the aim is ultimately to identify individuals in which decreased DNIC efficacy contributes to the pain phenotype and address this mechanism therapeutically.[3] Determining a normal "range" of DNIC responses in dogs will require the study of additional numbers of dogs of a wider demographic, particularly in view of the inconsistently reported sex[32] and age[25] differences associated with CPM in man.

In conclusion, we have demonstrated a number of neurophysiological changes indicative of CS processes in dogs affected by spontaneous OA, consistent with findings in man. However, measurement of electrical thresholds seemed not to be a suitable parameter for CS using the current methods. The mechanisms involved may encompass both upregulation of nociceptive afferent pathways,[26] in addition to alterations in the balance of descending modulatory mechanisms as shown here. Increasingly, it seems that the pathophysiological mechanisms of human OA[21] are shared by the spontaneous disease in dogs, further validating canine spontaneous OA as a model for the human disease[31,38] and supporting the use of dogs for mechanistic clinical trials to advance therapeutic development in humans.

Conflict of interest statement

The authors have no conflict of interest to declare.

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Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/A622.

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2.5 General discussion

We identified that buprenorphine was the most widely available opioid in paper 2, consistent with the findings of other authors (Brodbelt, 2006), however the evidence from paper 1 suggested that methadone represented a more efficacious analgesic in dogs undergoing orthopaedic surgery. This finding could potentially represent suboptimal management of perioperative pain and therefore, based on the methods developed in Paper 1, we have undertaken further work comparing the efficacy of methadone with buprenorphine in dogs and cats undergoing ovariohysterectomy (Shah et al., 2018b; Shah et al., 2018a). This work also suggested that methadone demonstrates greater efficacy compared with buprenorphine in both of these surgeries, with the caveat that NSAIDs were administered at the conclusion of the assessment period, in contrast to the study design used in Paper 1. The finding of superior efficacy of the authorised dose of methadone compared to that of buprenorphine was important to disseminate to first opinion veterinary surgeons and was communicated by the head of the Bristol Anaesthesia and Analgesia Research Group, Dr. Jo Murrell, during a series of lectures delivered for Dechra[5]. Further surveys on prescribing practice of veterinary surgeons would be hoped to demonstrate an increasing prescription of methadone for perioperative analgesia. A question remains as to the the most appropriate statistical handling of Short Form Glasgow Composite Pain Scale data in longitudinal research studies, where animals are unable to walk owing to excessive sedation at some time points. A comparison of agreement between the two different

[5] https://www.dechra.co.uk/news/archive-news/pain-management-cpd-sessions-hailed-a-success?PID=5774&M=NewsV2&Action=1&currentPage=4 accessed February 16, 2020.

suggested methods for completing the GCPS-SF (converting to a decimel or scoring a maximum for all dogs that are too sedated to stand) with a contemporaneously assessed DIVAS may yield further information on the relative merits of each approach.

The increase in prescription of perioperative analgesics provides benefits in terms of improved pain management, but increases the number of animals exposed to drugs and potentially at risk for associated adverse events. The limitations of current passive adverse event reporting in animals are discussed in paper 3 and it is to be hoped that whilst we were unable to provide reliable estimates for the incidence of adverse events related to NSAID treatment, the paper raised awareness of the knowledge gaps in this area. Whilst we were able to quantify the frequency of NSAID associated AEs that were reported to the VMD there remains an urgent need for more accurate evidence regarding the incidence and severity of NSAID associated AEs (Belshaw et al., 2016). A better estimation of the incidence of AEs across a representative population might be achievable through data collation initiatives such as VetCompass™, though there are currently no projects listed on the VetCompass™ webpages with this aim[6]. Whilst NSAIDs are reportedly effective in managing canine osteoarthritis pain (Sanderson et al., 2009), the cohort of dogs treated with NSAIDs in paper 5 continued to show evidence of augmented nociceptive processing. The electrophysiological demonstration of central sensitisation associated with osteoarthritis, despite NSAID treatment, provides the basis to investigate the effects of centrally acting analgesics and anti-hyperalgesics on spinal nociceptive processing. Additionally our described technique to elicit DNIC in anaesthetised dogs obviates the clear ethical and

[6] https://www.rvc.ac.uk/vetcompass/projects?filter=15703,15724 accessed February 16, 2020.

welfare concerns of delivering moderately painful stimuli over a period of time to a conscious animal that is unable to appreciate the reason for the application of the stimulus. Given the findings of normalisation of CPM in conscious dogs treated with cimicoxib (Monteiro et al., 2018) it would be informative to evaluate the effect of this drug on DNIC responses in our model.

3. Contributions to Other Research and Communication of Research Findings.

The expertise that I developed during the clinical research on acute pain meant that I was invited to contribute to the design and manuscript writing of the research project "A non-inferiority trial comparing paracetamol / codeine and meloxicam for post-operative analgesia in dogs", which has recently been accepted for publication (Pacheco et al. 2020). Data relating to physiological measures recorded during alfaxalone total intravenous anaesthesia in dogs (Paper 5) have been published, increasing the evidence base for the use of this technique clinically in middle aged to older dogs (Hunt et al., 2019a).

During the BBSRC research project I was able to act as MSc co-supervisor for Megan Goff, and we collaborated on the research project "Docosahexaenoic acid for the treatment of joint pain in dogs with osteoarthritis: A randomised, double-blinded, placebo-controlled trial", which has been submitted for publication to The Veterinary Journal. During my work in the field of analgesia I have been invited to produce articles on pain assessment and management for small animal veterinary surgeons (Hunt, 2014b, Hunt, 2014a), co-author

review articles on the current state of knowledge regarding QST in dogs (Hunt et al., 2019b), and co-author the chapter on chronic and osteoarthritic pain in the BSAVA Guide to Pain Management in Small Animal Practice (White & Hunt, 2019). I was invited to deliver a lecture “An update on opioids”, and join fellow speakers Samantha Lindley and Louise Clark in a panel presentation of pain management cases at the Association of Veterinary Anaesthetists residents day in March 2017.

4. Future Research.

I am currently working full time in clinical practice and am committed to continuing to remaining involved in research in companion animal pain management. Ethical review has been granted by the RCVS for a prospective, randomised, placebo controlled trial of palmitoylethanolamide for adjunctive analgesia in dogs undergoing hemilaminectomy, employing validated pain scales and mechanical nociceptive threshold testing, together with total opioid consumption as outcome measures. Data collection was planned to begin in early 2020, but is currently delayed by changes in working patterns resulting from coronavirus related movement restrictions.

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